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INHERITANCE OF SMOOTH SEEDS IN COTTON[1]

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INTRODUCTION

The presence or absence of fuzz or short hairs on the seeds of the very long staple commercial cottons is of practical interest because the separation of the lint from the seeds by the roller gin used in ginning these cottons is more difficult when the seeds are fuzzy than when they are smooth. Sea-island cotton, for which the roller gin was first used, has seeds almost devoid of fuzz, while the longest linted varieties of the Egyptian type (Sakellaridis in Egypt and Pima in Arizona) have relatively fuzzy seeds. Experience has shown that the speed of ginning is greater and the appearance of the cotton as it comes off the rolls is better in the case of a smooth-seeded than in the case of a fuzzy-seeded cotton. The development of smooth-seeded varieties of the very long staple cottons is therefore an objective of no small importance. As a guide to practical breeding work, an understanding of the inheritance of the characters dealt with is of course desirable. The purpose of this paper is to present such information as is available concerning the inheritance of smooth seededness in cotton.

Inheritance of the seed-coat character in a hybrid between an upland cotton (Holdon variety) and an Egyptian cotton (Pima variety) has been studied by one of the writers (*8, p. 11, 26, fig. 41*),[2] who also presented (*8, p. 32–33*) a brief summary of the results obtained by Fletcher (*6*), Fyson (*7*), Balls (*2, 3*), and McLendon (*11*). The only subsequently published contributions to our knowledge of the subject of which the writers are aware are those of Thadani (*12, 13*). He found (*13*) that in six crosses of a naked-seeded form of upland cotton with fuzzy-seeded upland varieties the fuzzless condition was completely dominant in F_1 and the F_2 segregation was monohybrid. In crosses between upland varieties distinguished by a greater or less degree of fuzziness, the less fuzzy condition appeared to be dominant, this being contrary to the conclusion of Balls (*2*) and McLendon (*11*). On the other hand, crosses of completely fuzzy upland cottons with partly fuzzy Egyptian cottons showed dominance of the more fuzzy (upland) condition in F_1, with F_2 distributions indicating that several factors are involved.[3] An exception was noted in a cross of a completely fuzzy-seeded upland with the nearly smooth-seeded Yuma variety of Egyptian cotton, the nearly smooth-seeded

[1] Received for publication Mar. 30, 1927; issued September, 1927. The investigation which is the principal subject of this paper was conducted at the United States Field Station, Sacaton, Ariz. The results obtained from crosses made at Greenville, Tex., by W. W. Ballard, of the Office of Cotton, Rubber, and Other Tropical Plants, also are considered on the basis of data furnished by Mr. Ballard. The photographs from which the illustrations were prepared were made by Robert L. Taylor of the same office.

[2] Reference is made by number (italic) to "Literature cited," p. 217.

[3] The results were therefore like those obtained by Kearney (*8*) with a similar cross.

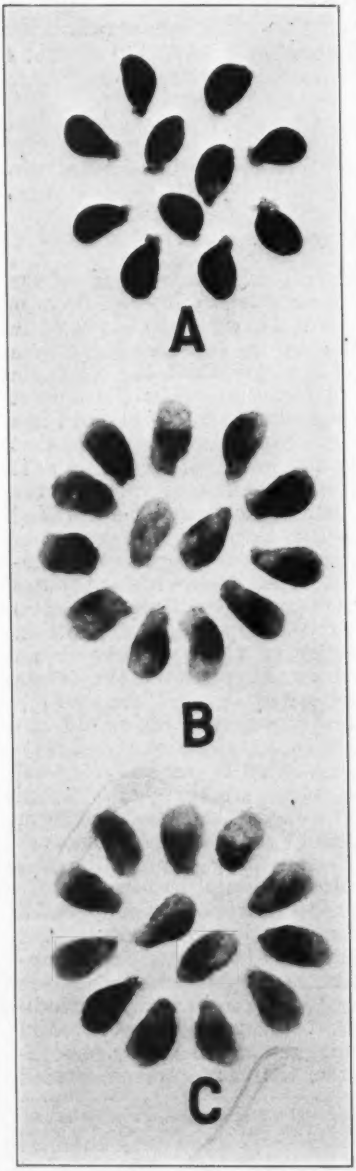

FIG. 1.—Seeds of the Pima parental families: A, the smooth-seeded family (P–88–7) with fuzz greatly reduced and sharply localized; B, a moderately fuzzy-seeded family (P Parker); C, a very fuzzy-seeded family (P spotless)

condition having been dominant in F_1. The F_2 segregates of this cross ranged from "absolutely naked" to "entirely fuzzy."

INHERITANCE OF SMOOTH SEEDEDNESS IN THE PIMA VARIETY

Excellent material for a study of the inheritance of presence and absence of fuzz on the seeds in the Pima variety of Egyptian cotton is afforded by a smooth-seeded family (Pima 20–SS–7) which uniformly has the seeds naked except for a small tuft of short fuzz at base and apex and occasionally a trace along the raphe, and a "spotless" family which has the seeds almost completely covered with fuzz, representing about the extreme of fuzziness in the Pima variety. The average quantity and distribution of the fuzz in the two parental families is illustrated in Figure 1, A and C.

In 1923 two plants (designated A–12 and A–13) in the homozygous smooth-seeded Pima progeny 7–2–10, and two plants (designated B–8 and B–12) in the homozygous smooth-seeded Pima progeny 7–2–11, were chosen as female parents. Flowers on these plants were emasculated and pollinated with pollen from one plant in each of the two homozygous "spotless" and fuzzy-seeded Pima progenies, 20–19 and 21–22.[4] The male parent in progeny 20–19 was designated H and the male parent in progeny 21–22 was designated J. The combinations were made as follows:

A 12 × H	A 13 × J
B 8 × H	B 12 × J

Each combination was represented by an F_1 progeny grown in 1924, and flowers were selfed on several plants in each of these progenies.

[4] These were F_3 progenies, recessive for petal spot and uniformly fuzzy seeded, which were derived from crosses of "spotless" with normally spotted Pima families, as described by one of the writers in another paper (9).

From the resulting seed 17 F_2 progenies were grown in 1925. These were:

A × H– 4	A × J– 3	B × H–1	B × J–12
A × H– 6	A × J– 4	B × H–3	B × J–13
A × H– 7	A × J– 9	B × H–4	B × J–14
A × H–21	A × J–18	B × H–5	B × J–15
		B × H–7	

Flowers were selfed on numerous plants in the F_2 progenies, and from the resulting seed 30 F_3 progenies were grown in 1926. These were:

A × H 4– 3*	A × H 21–22*	B × H 5– 1
A × H 4– 7*	A × H 21–26	B × H 5– 7*
A × H 4–24	A × J 4–15	B × J 12–11
A × H 6– 4*	A × J 9– 1*	B × J 13–19
A × H 6–19	A × J 9–12	B × J 13–23*
A × H 7–14*	A × J 18– 5	B × J 14– 2
A × H 7–15*	A × J 18–23	B × J 14–21
A × H 7–19	B × H 1–14	B × J 14–23
A × H 7–21	B × H 3–10*	B × J 15–11
A × H 21– 1	B × H 3–11	B × J 15–27

Ten of these progenies (indicated by asterisks) had a fuzzy-seeded F_2 parent, while the other 20 progenies had a smooth-seeded parent.

DOMINANCE OF SMOOTH SEEDEDNESS IN F_1

The whole population numbered 92, the four F_1 progenies containing from 21 to 24 plants each. All of these plants were similar to the smooth-seeded parent in the quantity and distribution of the fuzz on the seeds. In other words, the approximately naked condition of the seed coat of the smooth-seeded strain was completely dominant to the very fuzzy condition characteristic of the other parent of the cross.

SEGREGATION IN F_2

The individual plants of the F_2 progenies were readily classified as "smooth" or "fuzzy," since in nearly all cases their seeds were either as nearly naked as in the smooth-seeded parental family (fig. 1, A) or as fuzzy as in the fuzzy-seeded parent (fig. 1, C). The very few plants whose seeds showed appreciably more fuzz than has been observed in the former family, although less than is characteristic of the latter family, were classified as "fuzzy." The number of such individuals was far too small to affect the result.

The segregation in F_2 is shown in Table 1. Since the numbers of plants in the several progenies were small, all progenies of each combination (A × H, etc.) are taken as one array in presenting the results.[5] It is obvious that the segregation is monohybrid, the fuzzy-seeded condition behaving as a simple recessive. The departure of the percentage of fuzzy-seeded individuals from the expected 25 per cent in no case amounted to more than 2.6 times the probable error of the departure, and for the whole F_2 population of 381 plants taken as one array the departure was only 1.8 times its probable error.

[5] For the 17 F_2 progenies, χ^2 of the departure of the observed distribution from the distribution expected on the basis of a 3 : 1 ratio ranged from 0 to 5.4 the latter value indicating a chance of 1 in 50 that the departure was fortuitous. Only four of these progenies gave a χ^2 greater than 0.7.

TABLE 1.—*Segregation in respect to the seed-coat character in F_2 of crosses between a smooth-seeded and a fuzzy-seeded family of Pima cotton*

Combination	Number of progenies	Number of plants			Percentage of fuzzy-seeded plants
		Total	Smooth seeded	Fuzzy seeded	
A × H	4	102	74	28	27.4±2.98
A × J	4	99	66	33	33.3±3.19
B × H	5	72	56	16	22.2±3.34
B × J	4	108	80	28	25.9±2.84
Total	17	381	276	105	27.5±1.54

INHERITANCE OF CHARACTER IN F_3

Progenies of 20 smooth-seeded and of 10 fuzzy-seeded F_2 individuals were grown in 1926. The 10 progenies of recessive F_2's contained from 8 to 15 plants each, the combined F_3 population from fuzzy-seeded F_2 plants numbering 110 plants, all of which had seeds similar in degree of fuzziness to the fuzzy-seeded parental race. The recessive nature of the fuzzy condition is therefore completely established.

Of the 20 F_3 progenies of smooth-seeded F_2 plants, only two (A × J 18–23 and B × H 5–1) contained no fuzzy-seeded individuals, and the numbers were too small (17 and 19, respectively) to make it certain that they were homozygous for smooth seededness, especially since two other progenies of smooth-seeded F_2's, containing 19 and 20 plants, respectively, each included only one fuzzy-seeded individual. Assuming, however, that the progenies in which there were no fuzzy-seeded plants were really homozygous, it is to be noted that there were only two instead of the expected $20 \div 3 = 6.67$ such progenies. From the value of χ^2 (4.9) it appears that the chances are 1 in 37 that the departure from the expected number of homozygous progenies is fortuitous.

TABLE 2.—*Segregation in respect to the seed-coat character in 18 heterozygous F_3 progenies of crosses between a smooth-seeded and a fuzzy-seeded family of Pima cotton*

Combination	Progeny	Number of plants			Percentage of fuzzy-seeded plants	χ^2	P
		Total	Smooth seeded	Fuzzy seeded			
A × H	4–24	19	12	7	36.8	1.421	0.233
	6–19	18	16	2	11.1	1.852	.174
	7–19	20	14	6	30.0	.267	.605
	7–21	18	13	5	27.8	.074	.786
	21–1	19	18	1	5.3	3.948	.047
	21–26	22	17	5	22.7	.060	.806
A × J	4–15	16	9	7	43.7	3.000	.083
	9–12	17	10	7	41.1	2.372	.124
	18–5	16	13	3	18.7	.333	.564
B × H	1–14	19	10	9	47.3	5.070	.025
	3–11	21	17	4	19.0	.396	.529
B × J	12–11	20	14	6	30.0	.267	.605
	13–19	16	11	5	31.2	.333	.564
	14–2	20	19	1	5.0	4.267	.039
	14–21	19	13	6	31.5	.439	.508
	14–23	19	14	5	26.3	.017	.897
	15–11	19	17	2	10.5	2.122	.145
	15–27	19	17	2	10.5	2.122	.145

The segregation in the 18 heterozygous F_3 progenies is shown in Table 2. Since the progenies were small and in some of them the percentage of fuzzy-seeded individuals was very low, it seems best to judge the goodness of the fit by the probability, as indicated by χ^2, of the departure of the observed distribution from that expected with a 3:1 ratio. The chance of a fortuitous departure from the expectation as great as that shown by the progeny which gave the highest value for χ^2 (5.07) is 1 in 40.

In Table 3 the several heterozygous F_3 progenies of each combination are taken as one array and the percentages of fuzzy-seeded plants are given for these larger populations. In none of them is the departure from the expected 25 per cent equal to twice its probable error, and for the entire heterozygous F_3 population the departure is only one-fourth its probable error.

TABLE 3.—*Segregation in respect to the seed-coat character in groups of heterozygous F_3 progenies representing each of the crosses between a smooth-seeded and a fuzzy-seeded family of Pima cotton*

Combination	Number of progenies	Number of plants			Percentage of fuzzy-seeded plants
		Total	Smooth seeded	Fuzzy seeded	
A × H	5	94	73	21	22.3±2.91
A × J	4	71	49	22	31.0±3.74
B × H	2	40	27	13	32.5±5.09
B × J	7	132	105	27	20.4±2.37
Total	18	337	254	83	24.6±1.58

INHERITANCE OF SMOOTH SEEDEDNESS IN CROSSES AMONG VARIOUS FAMILIES

Further indications of the manner of inheritance of the smooth and fuzzy condition of the seed coat were obtained by nine combinations made in 1924 among various families, some of which represented different species (Egyptian and upland cottons). With one exception (combination 3), each combination was represented by two crosses, different individuals of the respective parental families having been used as parents of each cross, except that in combination 8 the same Ballard individual served as the male parent of both crosses. F_1 progenies representing both crosses of each combination were grown in 1925. From selfed seed of a single individual in one of the F_1 progenies, an F_2 progeny of each combination was grown in 1926.

Five open bolls were collected from each plant in the parental and F_2 progenies of 1926, and the seed cotton was ginned with care to remove the lint as fully as possible without disturbing the fuzz. The seeds, freed from adhering lint, neps, and trash, were placed in transparent glass vials, one for each plant, and with the samples in this convenient form classification of the populations was readily accomplished.

CHARACTERISTICS OF THE PARENTAL FAMILIES

Of the parental families involved in these crosses, the first three herein described were of the Pima variety of Egyptian cotton (*Gossypium barbadense* × ?) and the other four were upland cottons (*G. hirsutum*).

PIMA SMOOTH SEED (P–SS–7)

The parental progeny was of the third inbred generation from a plant of the Pima variety selected at Sacaton in 1920 by Walter F. Gilpin and characterized by the almost complete absence of fuzz on the seeds. It proved to be homozygous for this character, no other kind of seeds having been found on hundreds of its descendants examined during the past six years. The particular individual which gave rise to the progeny grown in 1924 belonged to the progeny of 1923 (7–2–10) which furnished the "A" parents of the combinations described in the earlier pages of this paper. Different individuals in progeny 7–2–10–17 of 1924 were used as one of the parents of combinations 1, 2, 7, and 9, respectively. (Table 4.) Selfed seed of plant 5, the female parent of combination 7, was planted in 1926 and gave a progeny of eight plants. All of these were alike and showed the condition typical of this family, the fuzz being confined to a pronounced but sharply limited tuft at the small end of the seed and often a smaller tuft at the large end, with an occasional trace along the raphe. (Fig. 1, A.)

PIMA PARKER

The parental progeny was of the third inbred generation from an individual of the Pima variety selected by Warren Parker at Phoenix, Ariz. Different individuals in the progeny of 1924 (P. Parker 6–10–17) were used as one of the parents of combinations 1, 2, 6, and 8, respectively. (Table 4.) Selfed seed of plant 15, the female parent of combination 1, was planted in 1926 and gave a progeny of 10 plants, all of which had the seeds partly covered with typical Pima fuzz, greenish or brownish in color. (Fig. 1, B.) The average degree of fuzziness in this progeny was near the average for the Pima variety, the classification having been: Grade 6, 4 plants; grade 7, 4 plants; grade 8, 2 plants. As is the rule in the Pima variety, there was considerable variation on the individual plant in the degree of fuzziness.[6]

PIMA SPOTLESS

The parental progeny was a recessive F_4 of one of the crosses, described in another publication (*9*), between representatives of a Pima family having almost no petal spot and representatives of a normally spotted family of the same variety. It was grown from selfed seed of plant 34 in the F_3 progeny (21–22), which furnished the "J" parents of the combinations described in earlier pages of this paper. Both the spotless and the normally spotted parents of the original cross were fuzzy seeded, and the descendants have exhibited this condition uniformly. Different individuals in F_4 progeny 21–22–34 of 1924 were used as one of the parents of combinations 4 and 5, respectively. (Table 4.) An F_5 progeny grown in 1925 from selfed seed of another individual in the F_4 progeny comprised 18 plants, the seeds of all of which were decidedly more fuzzy (grade 8 to 9) than the average for Pima. Only two plants survived in the F_6 progeny grown in 1926, but these had seeds similar to those of the F_5 plants. (Fig. 1, C.)

BALLARD'S NAKED-SEEDED UPLAND

The writers are indebted to W. W. Ballard, the originator of this family, for the following record of its performance at Greenville, Tex. The original naked-seeded selection, in a population of upland cotton grown from seed obtained from Cuba, was made at Greenville in 1916. A progeny grown in 1917 from open-pollinated seed from this plant contained, as would be expected, some fuzzy-seeded individuals. Open-pollinated seed was saved from plants in the 1917 progeny which had naked seeds and very sparse lint. A progeny, grown in 1919 from seed of the most nearly lintless of the 1917 selections, contained some fuzzy-seeded plants and showed variation in the quantity of lint. Flowers were selfed on the plants grown in 1919, and selfed seeds from the plants most nearly lintless were used in planting three progenies grown in 1920. No fuzzy-seeded plants appeared in the 1920 progenies, and in one of them most of the plants were almost lintless. Selfed seed from two plants in the latter progeny gave rise to two progenies grown in 1921. Neither of these contained any fuzzy-seeded individuals.[7] Seed from a plant in one of these progenies was sent to

[6] In the type sample of grade 7 for Pima cotton the seeds have from about one-fourth to nearly all of the surface covered with fuzz.

[7] No record was preserved of the size of the progenies grown in 1920 and 1921, but it is Mr. Ballard's recollection that each of them contained about 20 individuals.

Sacaton, Ariz., and two sister plants in the same progeny were used as parents of crosses with two plants of Lone Star made by Mr. Ballard and hereafter described in this paper.[8] No fuzzy seeds appeared in two succeeding inbred generations of this family at Greenville, although the population grown in 1923 comprised 63 individuals.

The seed from Greenville was planted at Sacaton in 1924, and seven plants survived, all of which were recorded as having fuzzless seeds. (Fig. 2.) Different individuals in this population were used as one of the parents of combinations 3, 7, and 8, respectively. (Table 4.) From selfed seed of two of the plants, one of which (No. 1) had served as the Ballard parent of combination 3, progenies of four and five individuals, respectively, were grown in 1925. These plants were uniformly almost fuzzless, showing only a minute tuft of fuzz at the small end of the seed. Selfed seed of a plant (No. 1–2) in the 1925 progeny of plant No. 1 gave rise to a progeny of 10 plants grown at Sacaton in 1926.

The behavior at Greenville, rather than the absence of fuzzy seeds in the very small populations grown at Sacaton in 1924 and 1925, led the writers to believe that this family is homozygous smooth seeded, and on this assumption it was used in crosses with other families in 1924. It was a surprise, therefore, to find the 1926 Ballard progeny segregating in the proportion of seven naked or nearly naked to three fuzzy. The several types of segregates were exactly duplicated in F_2 of the cross Ballard × Lone Star (combination 3). The fuzzy-seeded individuals were very similar to Lone Star (fig. 3, A) having seeds completely covered with typical upland fuzz. (Fig. 4, D). Of the smooth-seeded segregates, two had approximately the quantity of fuzz (chiefly at the small end) characteristic of the smooth-seeded Pima family (fig. 4, C), while the other five had the seeds entirely or almost entirely devoid of fuzz (fig. 4, A and B).

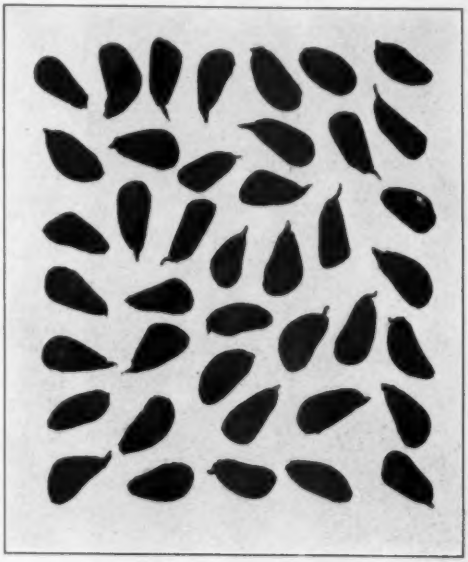

FIG. 2.—Seeds of the Ballard upland parental family, showing the typical fuzzless condition and the long slender funiculus or seed stalk

The possibility is not excluded, in spite of the precautions taken at Sacaton to avoid such errors, that seeds resulting from natural cross-pollination of unprotected flowers on plant No. 1 of 1924 may have been gathered accidentally with the selfed seeds of this plant and that a plant grown in 1925 from such a cross-pollinated seed was the mother of the 1926 progeny. This seems a reasonable explanation of the segregation observed in progeny 1–2 of 1926, because, as will be shown in discussing combination 3, of which plant No. 1 was the Ballard parent, the result in F_1 affords evidence that this plant was homozygous for smooth seeds.

Yet, although we may assume homozygosity of the particular individual of the 1924 Ballard progeny which gave rise to the segregating Ballard progeny of 1926, it will be seen from the results in F_2 of another cross (combination 7) that not all of the plants in the 1924 progeny of the Ballard family were homozygous. It is probable, therefore, that the seeds sent from Greenville and planted at Sacaton in 1924 included some which had resulted from accidental cross-pollination with a fuzzy-seeded upland cotton.

[8] It may be noted here that the F_1's of these crosses were uniformly smooth seeded, but the populations were too small to afford proof that the Ballard parents were homozygous for smooth seeds.

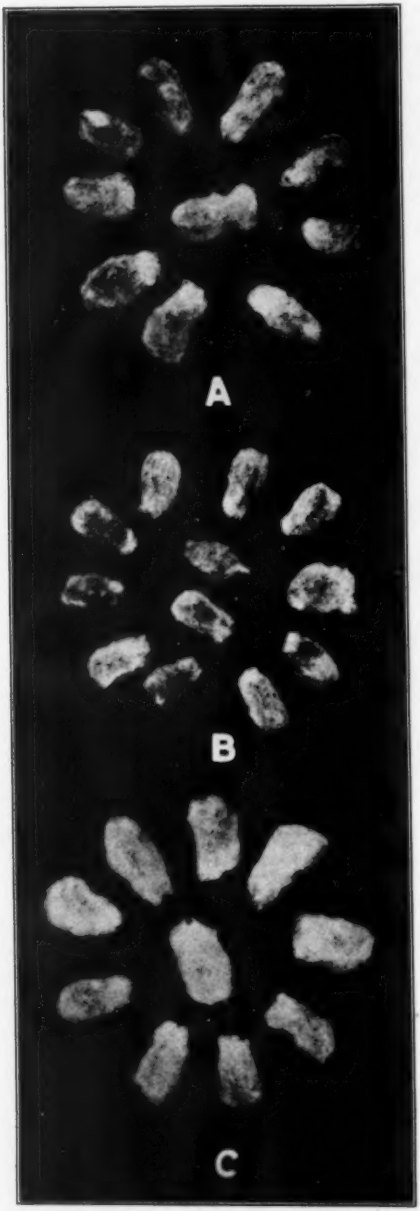

FIG. 3.—Seeds of the homozygous fuzzy-seeded upland parental families: A, Lone Star; B, Acala; C, Holdon

There is a tendency in this family, as is often the case in naked-seeded variations of upland cotton, to suppression of the long hairs or lint as well as of the shorter hairs or fuzz. (See fig. 10, p. 213.)[9] None of the smooth-seeded segregates in the 1926 progeny at Sacaton was entirely lintless, but some individuals had very little while others had fairly abundant lint. Another characteristic of the typical naked seeds (fig. 2) is the long and very slender stalk (funiculus). In this, as in other seed characters, the naked-seeded variants of upland cotton resemble the "Hindi" cotton which occurs as a weed in fields of cultivated cotton in Egypt (*5, p. 12*).

LONE STAR UPLAND

A population of eight individuals, from selfed seed of a plant grown at Sacaton in 1919, was grown in 1924. Different individuals in this progeny served as one of the parents of combinations 3, 6, and 9, respectively. (Table 4.) Selfed seed of another individual (No. 7) in this population gave rise to a progeny of nine individuals grown in 1925, and from selfed seed of one of the plants (7–4) in the 1925 progeny a progeny of nine individuals was grown in 1926. In all three generations all individuals showed the condition characteristic of the Lone Star variety and of most upland cottons, the seeds being covered with whitish fuzz much longer than that of Pima Egyptian. (Fig. 3, A.) The density of the fuzz varies, however, there being areas on some of the seeds of nearly all plants where the fuzz is so sparse that the dark seed coat shows through. On one plant of the 1926 progeny all of the seeds were thus characterized.

ACALA UPLAND

In a progeny of the third inbred generation, grown in 1924, one of

[9] In the segregates of combinations of which this family furnished one of the parents, it was not always easy to make a sharp distinction between lint hairs and fuzz hairs. The difference between the two kinds of hairs is stated by Balls (*4, p. 83*) as follows: "The hairs of the fuzz are distinguishable from those of the lint by their much greater diameter, even in the earliest stages of their development. They are as a rule about twice the diameter of a lint hair, or more. They arise in much the same way, at the same time, and from the same layer of cells."

the plants was used as the male parent of combination 4. (Table 4.) From selfed seed of the same individual (No. 22) a progeny of 11 plants was grown in 1925, and selfed seed of a plant in this progeny (22–5) gave rise to a progeny of 10 individuals grown in 1926. Both 1925 and 1926 progenies were homozygous for fuzzy seeds, a condition typical of the Acala variety. The fuzz, whitish in color, was shorter than in Lone Star and covered the seed completely, although usually less densely on the faces than on the ends of the seeds. (Fig. 3, B.)

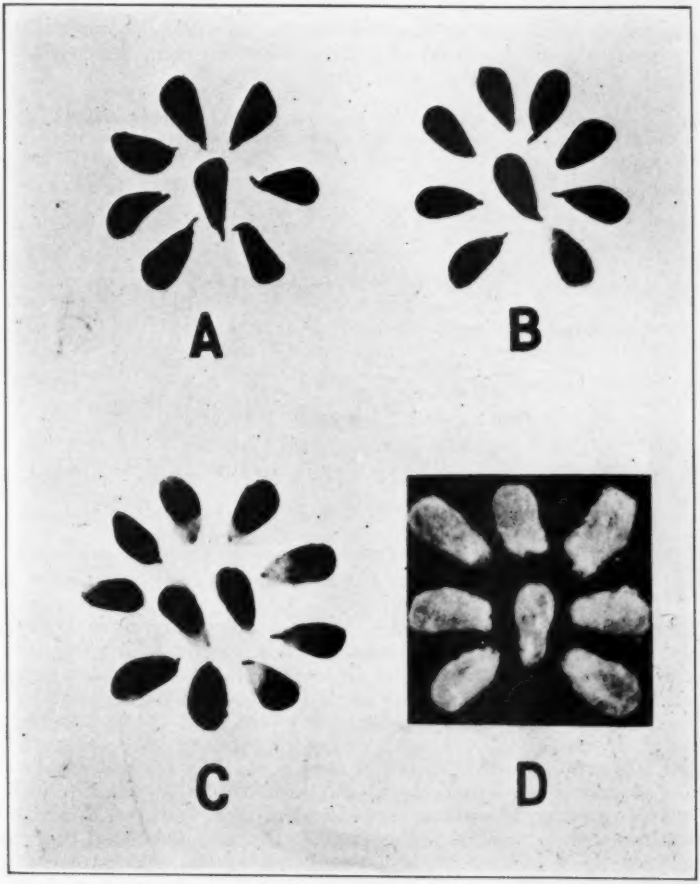

FIG. 4.—Seeds of F_2 plants of combination 3, a cross between a smooth-seeded upland (Ballard) and a fuzzy-seeded upland (Lone Star), showing the several types of segregates: A, fuzzless; B, nearly fuzzless; C, with localized fuzz, classed as smooth seeded; D, completely fuzzy

HOLDON UPLAND

No record was made of the seed coat condition in the progeny of 1924 which furnished the male parent of combination 5 (Table 4), but it was of the fifth inbred generation, and its ancestors were uniformly fuzzy seeded. The 10 plants of a progeny grown in 1926 were typical in having the seeds completely covered with nearly pure white fuzz, much longer and denser than in the Lone Star and Acala parental families. (Fig. 3, C.)

Comparing the several parental families, it will be noted that the seeds of the fuzzy-seeded uplands (fig 3) are more completely covered

and have longer fuzz than the seeds of the fuzzy-seeded Pimas (fig. 1, B and C). On the other hand, the seeds of the Ballard upland family (fig. 2) are more nearly naked than the seeds of the smooth-seeded Pima family (fig. 1, A).

BEHAVIOR OF THE CHARACTER IN THE HYBRID POPULATIONS

The results in F_1 and F_2 are presented in Table 4. The hybrid populations were too small to afford conclusive evidence of the manner of inheritance of this seed character, but the data are useful as suggesting lines of future investigation.

TABLE 4.—*Inheritance of the seed-coat character in various combinations of Pima Egyptian and upland cottons*

Character of seed coat of individuals which served as parents of the crosses	Combination	F_1[a]		F_2[b]		
		Smooth	Fuzzy	Smooth	Fuzzy	(?)
Smooth × fuzzy (Pima × Pima).	(1) P-SS-7 × P Parker	7	0	19	7	
		6	0			
	(2) P Parker × P-SS-7	8	0	15	6	
		8	0			
Smooth × fuzzy (upland × upland).	(3) Ballard × Lone Star	12	0	22	5	
Fuzzy × fuzzy (Pima × upland).	(4) P spotless × Acala	0	7	0	25	1
		0	9			
	(5) P spotless × Holdon	0	5	0	21	
		0	9			
	(6) P Parker × Lone Star	0	9	0	23	
		0	8			
Smooth × smooth (Pima × upland).	(7) P-SS-7 × Ballard	8	0	20	3	
		10	0			
Fuzzy × smooth (Pima × upland).	(8) P Parker × Ballard	10	0	12	9	1
		9	0			
Smooth × fuzzy (Pima × upland).	(9) P-SS-7 × Lone Star	0	10	2	11	1
		0	10			

[a] Except in combination 3, two F_1 progenies, representing crosses between different individuals of the same parental families, were grown in 1925.

[b] Each F_2 progeny was grown from selfed seed of a single plant in the F_1 progeny which is on the same line in the table.

Combinations 1 and 2 (Table 4) are reciprocal crosses between representatives of the smooth-seeded Pima family which furnished one of the parents of the crosses (A × H, etc.) described in the earlier pages of this paper and a fuzzy-seeded Pima family (P Parker) not related to the "spotless" family which furnished the fuzzy-seeded parents of crosses A × H, etc. The inheritance in combinations 1 and 2 is similar to that in the crosses previously described—dominance of smooth seededness in F_1 and monohybrid segregation in F_2.

In the quantity, character, and distribution of the fuzz, F_1 and the smooth-seeded segregates in F_2 were like the smooth-seeded parental family, P–SS–7 (fig. 1, A), and the fuzzy-seeded segregates in F_2 were like the fuzzy-seeded parental family, P Parker (fig. 1, B). The results suggest, although they do not prove, that the "Parker" family of Pima has the same major factor for seed fuzziness as the "spotless" family which furnished the fuzzy-seeded parents of combinations A × H, etc.

Combination 3 (Table 4) is a cross between a smooth-seeded family of upland cotton (Ballard naked seeded) and a family representing the typical condition in a very fuzzy-seeded variety of upland cotton (Lone Star). From presumably selfed seed of plant No. 1, the Ballard parent of this combination, four plants were grown in

1925, and all were smooth seeded; but a progeny of 10 plants grown in 1926 from selfed seed of a plant in the 1925 progeny segregated in the proportion of seven smooth seeded to three fuzzy seeded. Yet, as was stated in the account of the Ballard parental family, this segregation may have been due to accidental planting in 1925 of a seed resulting from natural cross-pollination on plant No. 1 of 1924, in which case it does not follow that the 1924 plant itself was heterozygous. That it was, on the contrary, homozygous is indicated by the fact that in F_1 of combination 3 all 12 of the plants were smooth seeded.[10] If the Ballard parent of this combination had been heterozygous, the Lone Star parent being homozygous recessive, half of the F_1 plants should have been fuzzy seeded. The value of χ^2 (*12*) indicates a chance of less than one in a thousand that the absence of fuzzy-seeded individuals in F_1 is fortuitous. The F_2 segregation suggests a ratio of three smooth to one fuzzy, the χ^2 of 0.6 indicating a chance of 1 in 2.3 that the departure of the observed distribution from the distribution expected with this ratio is fortuitous.

Of the 22 segregates in F_2, classed as smooth seeded, 18 had seeds that were completely fuzzless or very nearly so (fig. 4, A), three had seeds with minute tufts of very short fuzz, usually at the small end of the seed (fig. 4, B), and one had seeds with somewhat more fuzz than in the smooth-seeded Pima family (fig. 4, C). The fuzzy-seeded segregates resembled the Lone Star parent in the character and distribution of the fuzz. (Fig. 4, D.)

Combinations 4, 5, and 6 (Table 4) represent interspecific hybrids involving two fuzzy-seeded Pima families and three families, typical of as many varieties, of fuzzy-seeded upland. All of these families are believed to be homozygous recessive. One of the Pima families, P spotless (fig. 1, C), is the same involved in the crosses (A×H, etc.), described in the earlier pages of this paper, and the other, P Parker (fig. 1, B), was one of the parents of the reciprocal combinations 1 and 2. Of the three upland parental families, Acala had the least fuzzy seeds and Holdon the fuzziest seeds. (Fig. 3.)

In F_1 all individuals of the three combinations resembled the upland parent in the character and distribution of the fuzz. In F_2 of combination 4, 25 of the 26 plants had fuzz over the entire surface of the seed and many plants had much longer and denser fuzz than the Acala parent. The extremes of fuzziness among these 25 plants are shown in Figure 5, A and B.[11] In F_2 of combination 5 all of the 21 plants had the seeds covered with fuzz. On several plants the fuzz was shorter and less dense than on Holdon upland, the fuzzier seeded parent, while a few plants had even longer fuzz than Holdon. The extremes of fuzziness in this progeny are shown in Figure 5, C and D. In F_2 of combination 6, only 2 of the 23 plants had less fuzz than the upland parent (Lone Star), 1 plant having most of the seeds completely covered and the remaining seeds at least half covered, while the other plant resembled the Pima parent (P Parker) in the average quantity of fuzz present. Most of the remaining 21

[10] In addition to the F_1 progeny recorded in Table 4, two other F_1 progenies, representing the reciprocal combination Lone Star × Ballard, were grown in 1925 from crosses made in 1924 between other individuals of the same parental families. These comprised, respectively, nine and five plants, all smooth seeded.

[11] The exceptional plant had most of the seeds as smooth as or smoother than in the smooth-seeded Pima family (fig. 1, A), whereas a few of the seeds were comparable in fuzziness to those of the Pima Parker family fig. 1, B). The presence of this plant was almost certainly due to accident. This can not be proved, but it is highly improbable that a plant so nearly smooth seeded, alone in a progeny of 26, could have come from a cross between a fuzzy-seeded Pima and a fuzzy-seeded upland.

plants had longer and denser fuzz and several of them had much longer and denser fuzz than the fuzzier seeded (upland) parent. The range of seed fuzziness in this progeny is shown in Figure 6.

The parental phenotypes involved in combinations 4, 5, and 6 are similar to those involved in a cross between a very fuzzy-seeded upland family (Holdon variety) and a rather fuzzy seeded Egyptian family (Pima variety) described in an earlier publication (*8*). The seed fuzziness of the Egyptian parental family was similar to that of the

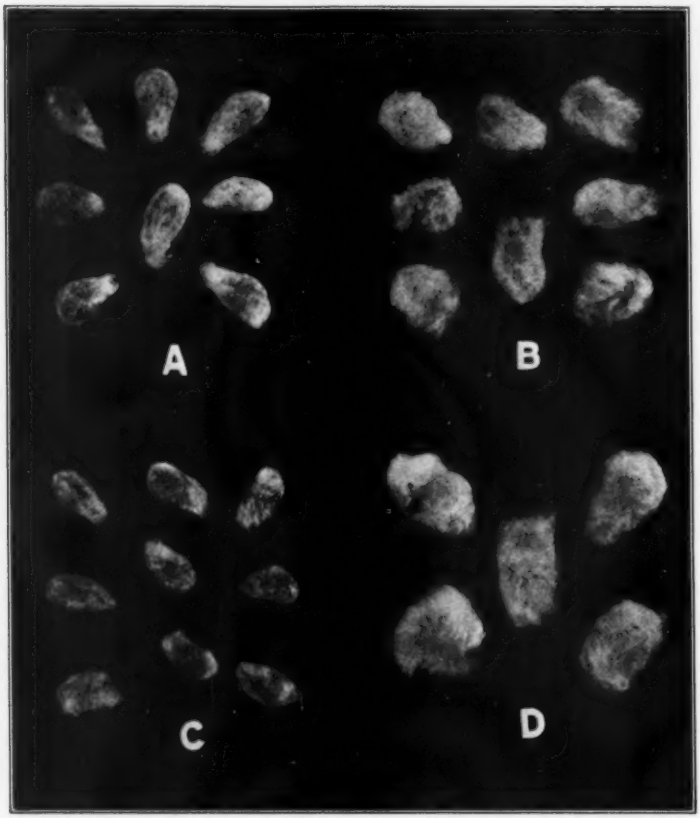

FIG. 5.—Seeds of F_2 plants of crosses between a fuzzy-seeded Pima and a fuzzy-seeded upland, showing the two extremes of fuziness: A and B, combination 4 (P spotless × Acala); C and D, combination 5 (P spotless × Holdon)

Pima family (P Parker) to which belonged one of the parents of combination 6, described in this paper. The upland parental family had all of the seeds covered with long white fuzz. In F_1 of the cross the seeds were approximately as fuzzy as in the upland parent, but the fuzz was green or pale brown in color. The frequency distribution of F_2, as given in the publication cited (*8, p. 26, fig. 41*), shows that a few individuals resembled the Pima parent in degree of fuzzi-

ness. The great bulk of the population was similar to F_1 and to the upland parent, and a number of plants exceeded the upland parent (fuzz much longer). Reexamination of this material confirms the conclusion that while no simple Mendelian ratio is determinable, the upland condition as to fuzziness is dominant over the Pima condition. In a total of 191 F_2 plants, only 7 resembled the Pima parent and none had smoother seeds. The seeds of 19 plants were

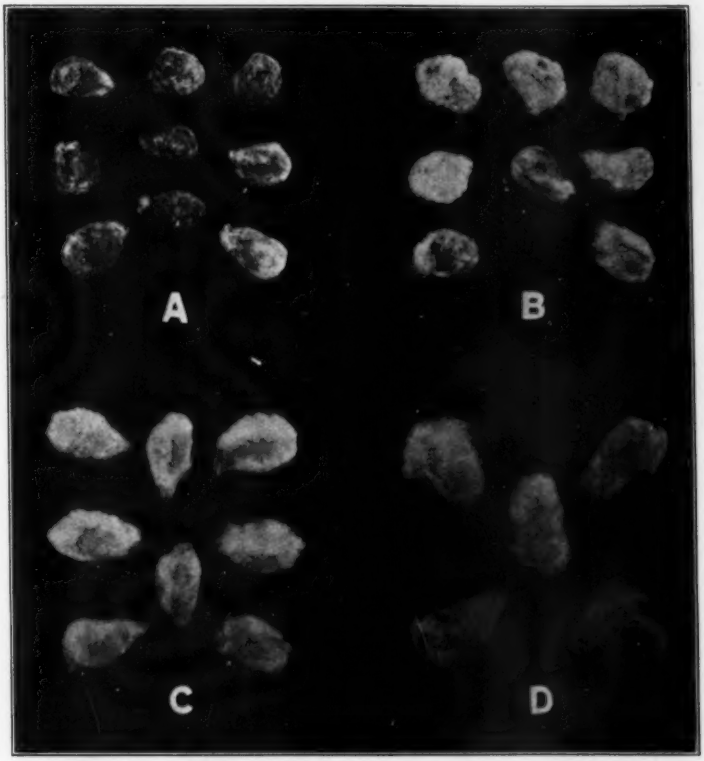

FIG. 6.—Seeds of F_2 plants of combination 6, a cross between a fuzzy-seeded Pima and a fuzzy-seeded upland (P Parker × Lone Star), showing the several types of segregates: A, the least fuzzy; B, less fuzzy than the average of the progeny; C, seed of average fuzziness; D, the most fuzzy

much fuzzier than the seeds of the Pima parent, although some of them were not completely covered. The remaining 165 plants had the seeds completely covered, and on many of these the hairs were longer than in the upland parent. There was also pronounced dominance of the greenish or brownish color of the Egyptian fuzz over the white color of the upland fuzz.[12]

[12] A large majority of the F_2 individuals showed the greenish or brownish fuzz color of the Egyptian parent as compared with the whitish color of the upland parent. Considering green alone, this color was apparent in 78.4 per cent of the F_2 population, while in 21.6 per cent the green color was not present, or at least was not apparent. The difficulty of classifying seeds in respect to color of the fuzz (*3, p. 146*) is a serious obstacle to the study of the inheritance of this color character.

Combination 7 (Table 4) represents an interspecific hybrid between a smooth-seeded Egyptian (Pima) family and the Ballard family of upland cotton. The individuals which served as the Egyptian parents belonged to the same family as the smooth-seeded

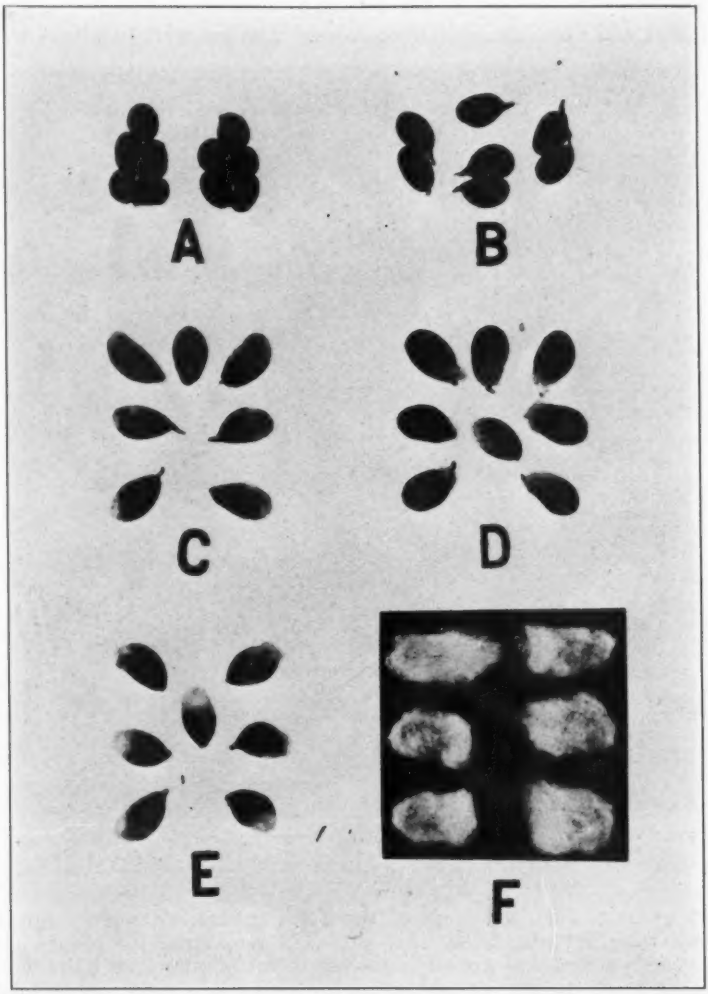

FIG. 7.—Seeds of F_2 plants of combination 7, a cross between the smooth-seeded Pima family (P-SS-7) and the smooth-seeded Ballard upland family, showing the several types of segregates: A, fuzzless, seeds strongly adherent; B, fuzzless, seeds weakly adherent; C, nearly fuzzless; D, with fuzz localized at the small end of the seed; E, with fuzz localized at the large end of the seed; F, completely fuzzy. (A to E were classed as smooth seeded)

parents of the Pima × Pima combinations A × H, etc., and 1 and 2 (Table 4) and were unquestionably homozygous. The individuals which served as the upland parents were phenotypically smooth

seeded. Whether they were homozygous or heterozygous was not determined by growing progenies from self-pollinated seed, and the absence of fuzzy-seeded individuals in F_1 does not answer the question, the Pima parents having been homozygous for smooth seeds. The segregation in F_2 (20 smooth, 3 fuzzy) and the uplandlike character of the fuzzy-seeded segregates indicate, however, that the Ballard parent of the corresponding F_1 progeny was heterozygous. The value for χ^2 of the departure from a 3:1 ratio in F_2 is 1.75, indicating a chance of 1 in 5.4 that the departure is fortuitous.

In F_1 all individuals were recorded as having the seeds completely devoid of fuzz. Of the 20 smooth-seeded segregates in F_2, 5 had the seeds entirely devoid of fuzz (fig. 7, A and B),[13] 12 showed the merest trace of fuzz (fig. 7, C), and 3 had a small tuft at either the large or the small end of the seed, the quantity of fuzz being about the same as in the smooth-seeded Pima family, although the hairs were longer (fig. 7, D and E). The three fuzzy-seeded segregates were similar to Lone Star in the quantity and distribution of the fuzz, but the hairs were longer than is usual in that variety. (Fig. 7, F.) It is interesting that the recessives of a hybrid between a smooth-seeded Pima and a phenotypically smooth-seeded upland showed even more than the normal upland degree of fuzziness. It is also to be noted that 17 out of 20, or 85 per cent, of the smooth-seeded F_2 segregates had the naked or very nearly naked seeds of the upland (Ballard) parent.

Combination 8 (Table 4) represents the interspecific hybrid Egyptian × upland in which the Egyptian (Pima) parents belonged to a fuzzy-seeded (homozygous recessive) family and the upland parent belonged to the Ballard family. The individual plant of the latter family used as the male parent in both crosses of this combination was phenotypically smooth seeded, but whether it was homozygous or heterozygous was not determined by planting self-pollinated seed. The fact that both F_1 progenies had the seeds uniformly devoid of fuzz suggests that the upland parent was homozygous, because if it had been heterozygous a 1:1 ratio should have appeared in F_1. The values for χ^2 of the departures from this ratio in the two F_1 progenies are 10 and 9, respectively, indicating chances of only about 1 in 640 and 1 in 370 that the departures are fortuitous. For the two F_1 progenies, as one array, $\chi^2 = 19$.

The observed segregation in F_2 of combination 8 (12 smooth, 9 fuzzy) throws no light upon the constitution of the upland grandparent, since the Egyptian grandparent having been recessive, the F_2 progeny of a phenotypically smooth-seeded F_1 plant should give a 3:1 ratio, whether the smooth-seeded (upland) grandparent was homozygous or heterozygous. The departure of the observed distribution in F_2 (omitting the one individual of doubtful classification) from the distribution expected with a 3:1 ratio gives a χ^2 of 3.57, indicating a chance of about 1 in 16 that the departure is fortuitous.

Of the 12 smooth-seeded F_2 segregates, 6 had the seeds devoid of fuzz or very nearly so (fig. 8, A), 5 showed minute tufts of fuzz

[13] Four of these five plants had some of the seeds adherent (fig. 7, A and B), although less tightly so than in typical "Kidney cotton" as described by Lewton (*10*). This character appeared in none of the other combinations described in this paper and was not observed in either of the families to which the parents of combination 7 belong. It is perhaps to be regarded as one of the remote ancestral characters which reappear in hybrids between different species of Gossypium (*8, p. 39–41*). The occurrence of "kidney" seed in a naked-seeded variant of upland cotton was noted by H. A. Allard (*1*).

(fig. 8, B), and 1 resembled the smooth-seeded Pima family in the quantity and distribution of the fuzz (fig. 8, C). The doubtful indi-

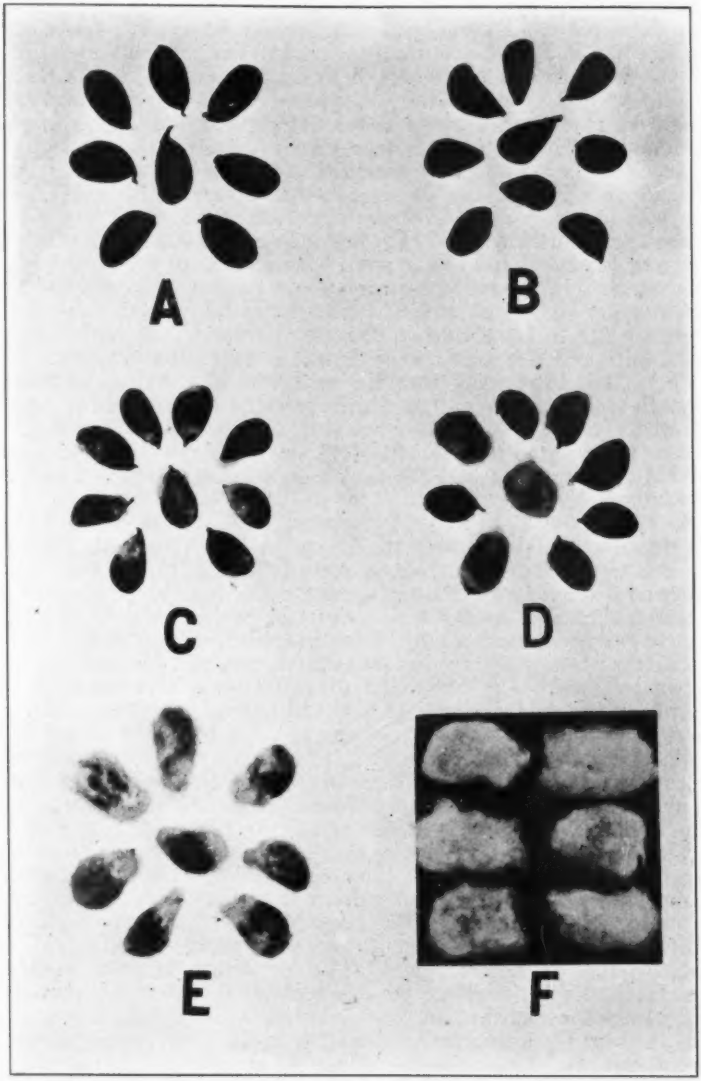

FIG. 8.—Seeds of F_2 plants of combination 8, a cross between a fuzzy-seeded Pima family (P Parker) and the smooth-seeded Ballard upland family, showing the several types of segregates: A, fuzzless; B, nearly fuzzless (most of the hairs showing are adherent lint hairs); C, having localized fuzz, but classed as smooth seeded; D, uncertain, having both fuzzy and nearly fuzzless seeds; E, least fuzzy of the fuzzy-seeded class; F, most fuzzy of the fuzzy-seeded class

vidual had some of the seeds devoid of fuzz and others more or less fuzzy. (Fig. 8, D.) Of the nine fuzzy-seeded segregates, eight had

all the seeds completely covered with uplandlike fuzz (fig. 8, F), but there was considerable variation from plant to plant in the length of the fuzz and in its color, which ranged from white to a rather vivid green. In the ninth fuzzy-seeded segregate all of the seeds had uplandlike fuzz (fig. 8, E), ranging in degree from half covered to completely covered.

The fact that the fuzzy-seeded segregates in F_2 of combination 8 resembled upland cotton rather than Pima in the quantity and length of the fuzz on the seeds points to the conclusion that the Ballard parent of this cross, while probably homozygous for the major factor which determines smooth seededness as contrasted with fuzzy seededness, also carried an intensifier for fuzziness which could express itself in F_2 only in those segregates which possessed the major factor for fuzziness contributed by the Pima parent of the combination.

Combination 9 (Table 4) represents the interspecific hybrid Egyptian×upland in which the Egyptian (Pima) parent belonged to the homozygous smooth-seeded family which furnished one of the parents of the crosses (A×H, etc.) described in the earlier pages of this paper, and of combinations 1 and 2 in Table 4; and the upland parent belonged to a typical family of the very fuzzy-seeded variety Lone Star. This combination affords the only indication of dominance of the fuzzy-seeded condition over the smooth-seeded condition obtained in this investigation. In the two F_1 progenies of 10 plants each, all were fuzzy seeded.[14] The segregation in F_2 was less clear cut than in the other combinations, but dominance of the fuzzy-seeded condition is strongly indicated, since 11 of the 14 plants had fuzzy seeds. Disregarding one plant of doubtful classification, the value for χ^2 of the departure of the observed distribution in F_2 from that expected with a ratio of 1 smooth to 3 fuzzy, is 0.64, indicating a chance of about 1 in 2.5 that the departure is fortuitous.

In both F_1 progenies many of the plants had seeds that were only partly covered with fuzz. Of the fuzzy-seeded segregates in F_2, six had the seeds completely covered (fig. 9, F), but there was considerable plant-to-plant variation in the length of the fuzz and in its color (whitish, brown, or green). Another F_2 plant had most of the seeds completely covered and the rest almost completely covered (fig. 9, E); whereas the remaining four fuzzy-seeded segregates ranged in degree of fuzziness from grade 5 to grade 9 of the Pima scale (fig. 9, C and D). The two F_2 plants classed as smooth seeded had somewhat more fuzz on the seeds than the smooth-seeded Pima family, corresponding to grade 2 of the Pima scale. (Fig. 9, A.) One plant in this F_2 progeny showed an anomalous condition. About half of the surface of the seed coat was sparsely covered with hairs which resembled short lint hairs, but there was a minute tuft of typical fuzz at the small end of the seed. (Fig. 9, B.) This plant probably belongs to the smooth-seeded class.

A comparison of the results from combinations 3 and 9 (Table 4) is particularly interesting. In combination 3 a phenotypically smooth-seeded and probably homozygous upland plant was crossed with a homozygous recessive (fuzzy-seeded) upland plant, while in combination 9 a homozygous smooth-seeded Egyptian plant was crossed

[14] This result is not in accord with that noted by Thadani (*15*), who states that in a cross of the comparatively smooth-seeded Yuma variey of Egyptian cotton with a fuzzy-seeded upland, the smoother seeded condition was dominant in F_1.

with a member of the same homozygous recessive upland family which furnished the fuzzy-seeded parent of combination 3. Dominance of the smooth-seeded condition is shown in combination 3 and domi-

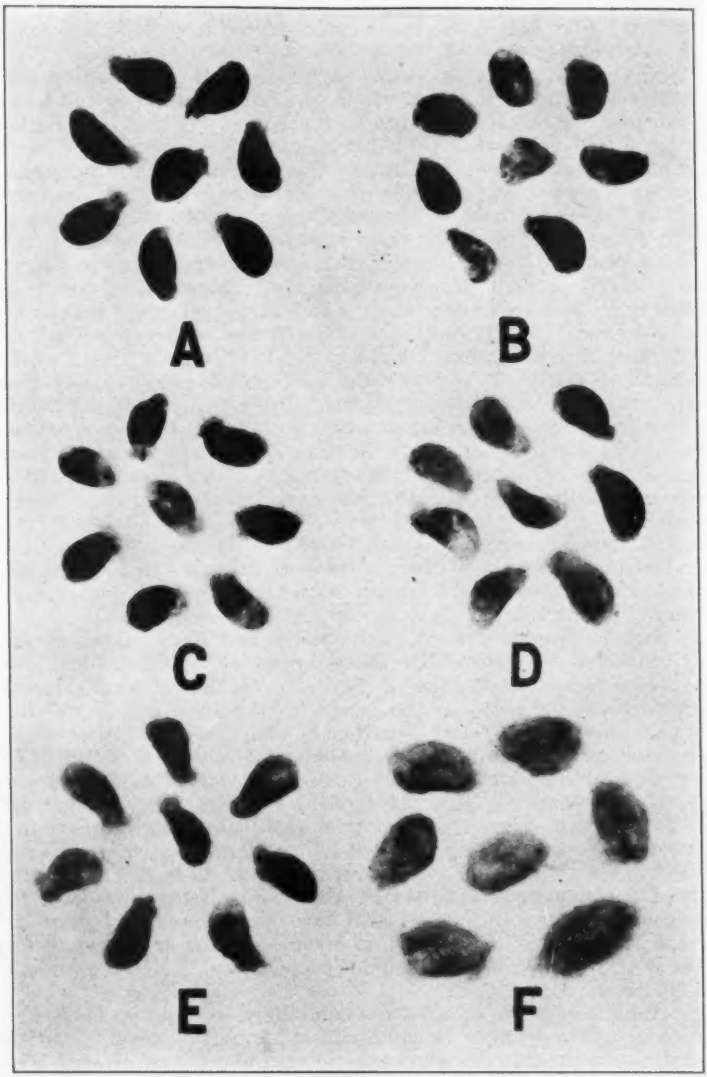

FIG. 9.—Seeds of F_2 plants of combination 9, a cross between the smooth-seeded Pima family (P-SS-7) and a fuzzy-seeded upland family (Lone Star), showing the several types of segregates: A, having localized fuzz, as in the Pima family, and classed as smooth seeded; B, nearly fuzzless, most of the hairs being adherent lint hairs; C and D, Pimalike in the character and distribution of the fuzz (classed as fuzzy seeded); E, completely or almost completely fuzzy seeded, fuzz short and sparse; F, completely fuzzy seeded, fuzz long and dense

nance of the fuzzy-seeded condition is shown in combination 9. Since there is no reason to doubt that the individual fuzzy-seeded parents of the two combinations were genetically identical in respect to this character, it would seem probable that the Ballard naked-seeded family of upland cotton and the smooth-seeded family of Egyptian (Pima) cotton have different factors for smooth seeds, the upland factor being dominant and the Pima factor recessive to the fuzzy-seed factor in Lone Star upland. This seems the less surprising in view of the fact that in the smooth-seeded upland family the fuzz normally is entirely wanting or reduced to a very slight trace (fig. 2), while in the smooth-seeded Pima family a pronounced though sharply localized tuft of fuzz is almost invariably present (fig. 1, A).

The conclusion that the factors for smooth seed in the two families are different might be regarded as weakened by the behavior of the cross between them (combination 7), since the uniformly smooth-seeded condition in F_1 suggests that the Egyptian and the upland parent had the same major factor for smooth seededness. On the other hand, the fact that 85 per cent of the smooth-seeded segregates in F_2 resembled the upland rather than the Egyptian parent in their naked or very nearly naked seeds, strengthens the assumption that the smooth-seeded forms of the two types of cotton have a different factorial constitution.

The factor for smooth seeds in Pima, although recessive to the factor for fuzzy seeds in Lone Star upland, is dominant to the factor for fuzzy seeds in Pima, as is shown clearly by the results with combinations A×H, etc., described in the earlier pages of this paper, and with combinations 1 and 2. (Table 4.) It may be recalled in this connection that the degree of fuzziness in the fuzzy-seeded Pima families (fig. 1, B and C), is always much less than in the Lone Star upland family (fig. 3, A).

It can not be determined from the results with combinations 4, 5, and 6 (Table 4) whether the fuzzy-seeded Pima and upland families differ in the major factor for seed fuzziness, although it is almost certain that the two types of cotton possess different modifying factors for this character. This supposition is favored by the occurrence of typical upland fuzz in some of the F_2 segregates of a cross between a fuzzy-seeded Egyptian plant and a presumably homozygous smooth-seeded upland plant (combination 8); and also by the fact that in crosses between a fuzzy-seeded Egyptian and a fuzzy-seeded upland (combinations 4, 5, and 6) many of the F_2 segregates exceeded the fuzzier seeded (upland) parent in the length and density of the fuzz. It also seems likely that the fuzzy-seeded upland parental families differ genetically. This is indicated by a comparison of the fuzziest seeded segregates of combinations 4 and 5. The same homozygous Egyptian (Pima) family furnished one of the parents of these combinations, but the upland parent was of the Acala variety in combination 4 and of the Holdon variety in combination 5. Holdon (fig. 3, C) has much longer and denser fuzz than Acala (fig. 3, B), and some of the F_2 segregates of combination 5 (fig. 5, D) had much longer and denser fuzz than the fuzziest seeded F_2 segregates of combination 4 (fig. 5, B).

LINKAGE RELATIONS

SEED FUZZINESS AND PETAL SPOT

The combinations described in the earlier pages of this paper (A×H, etc.) had as the fuzzy-seeded parents members of a Pima family characterized by almost complete absence of the large dark red petal spot which is of almost universal occurrence in the Egyptian type of cotton. The "spotless" condition has been shown to be recessive to normal development of the spot and to be determined by a single major factor (*9*). It will be of interest to note the inheritance of this character in relation to that of smooth seededness in the combinations dealt with in this paper.

The F_1 of combinations A×H, etc., grown in 1924, had the spot present on all plants, although it was less developed than in the normally spotted parent. This was in accordance with the results of previous observation (*9, p. 494*). Likewise, as in the earlier investigation (*9, p. 496*), the incompleteness of the dominance of spotted petal was shown in F_2. The dominance is so nearly complete, however. that separate classification of the homozygotes and heterozygotes of the spotted class is very difficult and was not attempted in considering the results in F_2.

Since spotless petal is recessive to the spotted condition and fuzzy seededness is recessive to smooth seededness, F_2 should exhibit a 9:3:3:1 ratio provided there is no linkage of the two genes and no differential survival among the several classes of gametes and zygotes.

In determining whether the two characters are linked, the procedure was as follows: The observed percentage of spotless individuals in F_2 having been 19.4 and the observed percentage of fuzzy-seeded individuals having been 27.6, the percentage of the spotless-fuzzy (double-recessive) class should have been 19.4 per cent of 27.6 per cent = 5.35 per cent. The expected percentages for the other classes, computed on the same basis, are: Spotted smooth (double dominant), 58.35 per cent; spotted fuzzy, 22.25 per cent; and spotless smooth, 14.05 per cent. The expected numbers in the several classes, corresponding to the expected percentages, as compared with the observed numbers, are shown in Table 5. The value for χ^2 (0.1628) indicates a very good fit, the chances being about 7 in 10 that the departure from the expectation is fortuitous. It may be concluded, therefore, that there is no linkage between the genes for presence of the petal spot and smoothness of the seed coat.

On the assumption of a 9:3:3:1 ratio, which would make the expected numbers for the several classes 214.3, 71.4, 71.4, and 23.8, χ^2 for the departure of the observed distribution is 8, indicating a chance of only 1 in 200 that the departure from the expectation is fortuitous. It is probable, therefore, that there was a small degree of differential survival of gametes or of zygotes, favoring the class with spotted petals at the expense of the spotless class. For the departure from the expectation in this case (three spotted to one spotless), χ^2 is 6.3, indicating a chance of only about 1 in 100 that the deficit of spotless individuals is fortuitous. There is also a smaller deficit of smooth-seeded individuals, but this departure probably is fortuitous, χ^2 being only 1.3.

TABLE 5.—*Percentages and numbers expected in the absence of linkage and numbers observed in the several classes in F_2 of crosses between a spotted smooth-seeded and a spotless fuzzy-seeded family of Pima cotton*

[Combinations A×H, A×J, B×H, B×J as one array]

Class	Percentage expected	Number	
		Expected	Observed
Spotted smooth	58.35	222.3	221
Spotted fuzzy	22.25	84.8	86
Spotless smooth	14.05	53.5	55
Spotless fuzzy	5.35	20.4	19
Total	100	381	381

SEED FUZZINESS AND ABUNDANCE OF LINT

Working with "American cotton," presumably upland, and crossing a smooth-seeded (fuzzless) form having sparse lint with a

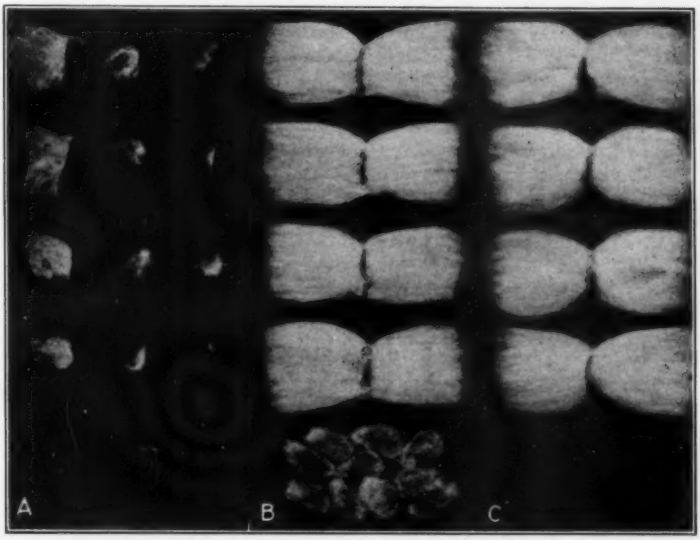

FIG. 10.—Seeds, before and after removal of the lint hairs, showing the dominance of smooth seeds and abundant lint in a cross between two upland cottons made by W. W. Ballard: A, the parent which had smooth seeds and sparse lint; B, the parent which had fuzzy seeds and abundant lint; C, F_1 which had smooth seeds and abundant lint

fuzzy-seeded form having abundant lint, Thadani (*12*) found smooth seeds and abundant lint to be dominant in F_1. In F_2 either character separately gave a near approach to a 3:1 ratio, whereas the distribution with respect to both characters was as follows: Smooth seeds and abundant lint, 103; smooth seeds and sparse lint, 54; fuzzy seeds and abundant lint, 53; fuzzy seeds and sparse lint, none. Thadani concluded that there is complete linkage of smooth seeds and sparse lint.

Similar crosses of two upland cottons were made in 1921 at Greenville, Tex., by W. W. Ballard, to whom the writers are indebted for use of the resulting data. The two parent individuals having smooth seeds and sparse lint were of the same Ballard family which furnished one of the parents of combinations 3, 7, and 8 described in this paper. The two parent individuals having fuzzy seeds and abundant lint were of the Lone Star variety. Both F_1 progenies showed complete dominance of smooth seeds and of abundant lint. Seeds, with and without lint, of the parents and of F_1 are shown in Figure 10. From selfed seed of an individual in each F_1 progeny, an F_2 progeny was grown at Greenville in 1923, the F_2 populations having comprised, respectively, 164 and 100 plants. As classified by Ballard, the distributions in F_2 were strikingly similar to the one recorded by Thadani. In this case also either character separately gave a near approach to a 3:1 ratio, and the double-recessive class was not represented.

In order to determine whether there is linkage of the two characters, the same procedure was followed as in dealing with seed fuzziness and petal spot. The percentages and numbers expected in case there were no linkages and the numbers observed in F_2 are shown in Table 6. Since χ^2 of the departure of the observed from the expected distribution amounts to 17.2 in progeny A and 8.2 in progeny B, the chances are very small that the departures are fortuitous; and it may be concluded that linkage of smooth seeds and sparse lint is complete or nearly so, as was the case in Thadani's material. Whether there was crossing over, and if so, in what proportion, can not be determined with certainty, because of the relatively small size of the F_2 populations and the fact that the double-recessive class was not represented.

TABLE 6.—*Percentages and numbers expected in the absence of linkage and numbers observed in the several classes in F_2 of crosses made by Ballard between a smooth-seeded sparse-linted and a fuzzy-seeded abundant-linted family of upland cotton*

Class	Progeny A			Progeny B		
	Percentage expected	Number		Percentage expected	Number	
		Expected	Observed		Expected	Observed
Smooth abundant	57.2	93.8	84	59.9	59.9	55
Smooth sparse	19.0	31.2	41	13.1	13.1	18
Fuzzy abundant	17.8	29.2	39	22.1	22.1	27
Fuzzy sparse	6.0	9.8	0	4.9	4.9	0
Total	100	164	164	100	100	100

SUMMARY

It is desirable that the seeds of long-staple cottons which are ginned on the roller type of gin should be as free as possible from fuzz or short hairs. This makes smooth seededness an important objective in breeding work. Knowledge of the manner of inheritance of smooth and fuzzy seeds will therefore be useful to cotton breeders.

This paper presents the evidence, from crosses between smooth-seeded and fuzzy-seeded cottons, that the inheritance of this character is mainly of a simple Mendelian type.

Crosses were made between representatives of a family of Pima Egyptian cotton homozygous for very little fuzz on the seeds, designated the smooth-seeded Pima family, and representatives of a family homozygous for the much more fuzzy condition of the seed coat typical of the same variety. F_1 was uniformly smooth seeded and the segregation in F_2 was in the ratio 3 smooth to 1 fuzzy. The smooth-seeded and the fuzzy-seeded segregates closely resembled the respective parents.

Third-generation progenies were grown from self-pollinated seed of 20 smooth-seeded and of 10 fuzzy-seeded F_2 plants. The progenies of fuzzy-seeded F_2's were uniformly fuzzy seeded. Of the smooth-seeded F_2 plants, two were probably homozygous, their F_3 progenies having contained no fuzzy-seeded plants. The remaining 18 progenies showed segregation in various proportions, but when taken as one array, 24.6 per cent of the population were fuzzy seeded, and the departure from the expectation (25 per cent) was only one-fourth its probable error. The evidence justifies the conclusion that in the Pima variety the fuzzy condition is recessive to the smooth condition.

The same and other smooth-seeded and fuzzy-seeded families also were used in another series of combinations. (Table 4.) Some of these were hybrids between very different species of Gossypium, Egyptian cotton (*G. barbadense* × ?) and upland cotton (*G. hirsutum*). The hybrid populations of these combinations were small, and the results obtained are to be regarded as suggesting rather than proving the nature of the inheritance.

Of the families which furnished the parents of these combinations, the smooth-seeded family of upland cotton had the seeds almost entirely devoid of fuzz, hence much smoother than in the smooth-seeded family of Egyptian (Pima) cotton. The fuzzy-seeded families of upland cotton, on the other hand, had much fuzzier seeds than the fuzzy-seeded families of Egyptian (Pima) cotton.

Crosses (combinations 1 and 2) between the smooth-seeded Egyptian (Pima) family used in the first series of combinations and another fuzzy-seeded Egyptian (Pima) family, not related to the family used in the first series, showed similar dominance of smooth seededness and monohybrid segregation in F_2.

The results of a cross between two plants of upland cotton, one phenotypically smooth seeded and probably homozygous, the other homozygous for fuzzy seeds (combination 3), indicated that in upland cotton also there is dominance of the smooth condition, and monohybrid segregation in F_2.

Crosses between fuzzy-seeded Egyptian (Pima) and fuzzy-seeded upland families (combinations 4, 5, and 6) produced only fuzzy-seeded plants in F_1 and, almost certainly, only fuzzy-seeded plants in F_2, the results indicating that all of these families were homozygous.

A cross between a homozygous smooth-seeded Egyptian (Pima) plant and a phenotypically smooth-seeded upland plant (combination 7) gave a uniformly smooth-seeded F_1 and a near approach to a 3 (smooth) to 1 (fuzzy) ratio in F_2. The occurrence in F_2 of fuzzy-seeded segregates which resembled upland cotton in the quantity and character of the fuzz, suggests that the individual of the

smooth-seeded upland family which served as one of the parents of this cross was heterozygous.

A cross between a homozygous fuzzy-seeded Egyptian (Pima) plant and a phenotypically smooth-seeded upland plant (combination 8) gave a uniformly smooth-seeded F_1 and segregation in F_2 in the proportion of 12 smooth to 9 fuzzy, which, because of the small size of the population, is probably not a significant departure from a 3:1 ratio. Although the absence of fuzzy seeds in F_1 indicates that the upland parent was homozygous in respect to the major factor for smooth seeds, yet this plant may have carried an intensifier for fuzziness, since the fuzzy-seeded segregates in F_2 had the upland degree of fuzziness.

A cross between a homozygous smooth-seeded Egyptian (Pima) plant and a homozygous fuzzy-seeded upland plant (combination 9) gave only fuzzy-seeded plants in F_1 and segregation in F_2 in approximately the ratio of 1 smooth to 3 fuzzy. In both hybrid generations there was considerably greater variation in the quantity and character of the fuzz than was the case with the other combinations.

The behavior of combination 9 indicates dominance of the upland factor for fuzzy seeds over the Egyptian factor for smooth seeds, whereas in combination 3 the upland factor for fuzzy seeds was recessive to the upland factor for smooth seeds. Since the same (doubtless homozygous) upland family furnished the fuzzy-seeded parents of both combination, it may be concluded that different factors for smooth seeds occur in Egyptian and in upland cotton.

It also seems probable that the slightly fuzzy condition of the smooth-seeded Egyptian is recessive to the naked or nearly naked condition of the smooth-seeded upland, since in the cross between a smooth-seeded Egyptian and a smooth-seeded upland (combination 7) 85 per cent of the smooth-seeded segregates in F_2 had the seeds naked or very nearly so.

The factor for smooth seeds in Egyptian (Pima), although recessive to the factor for fuzzy seeds in upland, is dominant to the factor for fuzzy seeds in Egyptian (Pima), as was shown in the earlier pages of this paper.

The occurrence of modifying factors or intensifiers for fuzziness is indicated by the results from crosses of fuzzy Pima with fuzzy upland (combinations 4, 5, and 6). That such a factor or factors may be carried by a plant homozygous for the major factor determining smoothness as contrasted with fuzziness is suggested by the results of the cross between fuzzy Pima and smooth upland (combination 8).

The crosses described in the early pages of this paper had for their parents members of a Pima Egyptian family homozygous for both smooth seeds and normally spotted petals and members of another Pima family homozygous for both fuzzy seeds and almost spotless petals. Complete independence of the characters, smooth and spotted, is demonstrated by the results from these crosses.

Crosses made by Ballard between members of a family of upland cotton having fuzzless seeds and sparse lint and members of an upland family having entirely fuzzy seeds and abundant lint gave satisfactory evidence of complete or nearly complete linkage of fuzzless seeds and sparse lint. The results confirm those which Thadani obtained with a similar cross.

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THE PRODUCTION OF CERTAIN ENZYMES BY BACTERIUM PRUNI[1]

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INTRODUCTION

Erwin F. Smith (*8, 9, 10, p. 57–60*)[2] observed that *Bacterium pruni*, studied and named by him, forms characteristic crystals when grown aerobically in skim milk. Upon request of Doctor Smith, the writer made a biochemical study of this disease-producing organism parasitic on plum and peach trees (*6, 8, 9, 10, p. 57–60*). The study led to the identification of the aforementioned crystals, which were shown to be made up of tyrosine, leucine, and higher fatty acids (*4*). Of these compounds tyrosine was identified by the xanthoprotein, Millon, and Mörner reactions and the melting point and nitrogen estimations, while leucine was identified by the form of its crystals, its ability to sublime, and its melting point. Certain properties of the third constituent of the crystals, namely, the light specific gravity, the ability to form a fatty spot on paper, and the melting point, have shown it to be a mixture of stearic, palmitic, and myristic acids. These were demonstrated to be present partly in the free state and partly in the form of a calcium salt, as shown by the ability of the latter to form, on distillation, the corresponding ketones, stearone, palmitone, and myristone.

PROTEOLYTIC AND LIPOLYTIC ENZYMES FOUND

While the primary object of identifying the crystals had thus been attained, it was realized that a consideration of the enzymes produced by the microbe would throw additional light on its metabolism. Because of the occurrence in milk of the proteins casein, lactoglobulin, lactalbumin, and opalisin (*1, p. 383; 5; 7*), attention was naturally directed first to the proteolytic enzymes. The proteases are known to have the ability to digest and to split up proteins of plant and animal origin into amino acids (*2, 11*). This was exactly the case when *Bacterium pruni* was grown in milk. Despite the fact that growth of the organism occurred at room temperature, and not at body temperature (37–38° C.), generally considered to be the optimum temperature for the activities of enzymes (*3, p. 521*), the amino acids tyrosine and leucine have definitely been shown to result in the metabolism of the milk proteins by *Bact. pruni*. That tyrosine and leucine, the well-known constituents of casein, lactoglobulin, and lactalbumin, were shown to be among the products of growth of the microbe can not be surprising when it is considered that they are difficultly soluble amino acids. That other amino acids which may

[1] Received for publication May 2, 1927; issued September, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 220.

have been present were not in evidence, was probably due to their greater solubility in the culture medium employed and perhaps also to the fact that because of their ready solubility they could be utilized by *Bact. pruni* for the structural process (building up its tissues).

The occurrence of butterfat in milk directed attention to the possible presence of lipolytic enzymes in the culture medium. Lipases are known to split fats into their component parts, glycerol and fatty acids (*2, p. 237*). This was true of the culture medium in which *Bact. pruni* was grown. Stearic, palmitic, and myristic acids were shown to be present in it. That the lower fatty acids, such as butyric, caproic, and caprylic, were not present in the crystals was probably due to their comparatively greater solubility in the nutrient medium. That a part of the identified acids referred to above has been found in the form of a calcium salt can not be surprising in view of the fact that calcium is known to be a normal constituent of milk.

Mention should be made of the fact that the skim milk in which *Bacterium pruni* was grown had been sterilized on four consecutive days for fifteen minutes each, at a temperature of 100° C. This heating of the milk, which practically has a neutral reaction, could not possibly have any disintegrating effect upon its native proteins. The latter, it will be remembered, must be boiled for several hours with *strong acids* or *alkalies* before they can be split up into amino acids. Nor could the butterfat undergo hydrolysis under the conditions mentioned. Furthermore, the heated milk was perfectly free from enzymes which, while present in the original milk, were completely destroyed by the process of sterilization. Consequently, the amino acids and fatty acids found in the milk must necessarily be ascribed to the action of the enzymes which were elaborated by the microbe upon the milk constituents.

SUMMARY

Proteolytic and lipolytic enzymes have been shown to be present among the products of growth of *Bacterium pruni* in milk.

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INHERITANCE OF WINTER HARDINESS AND GROWTH HABIT IN CROSSES OF MARQUIS WITH MINHARDI AND MINTURKI WHEATS[1]

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INTRODUCTION

Winter wheat usually matures earlier than spring wheat and thus often escapes the hot, dry winds and rust which frequently injure spring wheat. When winterkilling is not a factor, the yield of winter wheat is greater, as a rule, than that of spring wheat. Winter wheat has the advantage of aiding in the distribution of labor upon the farm. For these reasons it is generally grown instead of spring wheat wherever it proves successful.

The introduction of the hard red winter wheats into this country has resulted in a northward expansion of the area devoted to the culture of winter wheat. Low temperatures, combined with other environmental factors to which winter wheat often succumbs, have limited its wider use. The acreage of winter wheat abandoned annually in the United States, primarily as the result of winterkilling, varied from 1.1 to 28.9 per cent in the 25-year period from 1901 to 1925, inclusive. The average abandonment for the five years from 1921 to 1925 was 12.5 per cent, and in 1925 the estimated percentage was 21.7 (*1*).[2]

The development of more hardy varieties has made possible the successful growing of winter wheat in those areas where formerly it frequently was injured by winterkilling. As an illustration may be mentioned the variety Minturki, which was obtained by crossing Odessa, a late-maturing, winter-hardy variety, with Turkey. The new variety has proved very successful in southern Minnesota.

In addition to the inability of fall-sown wheat to survive the winter in the upper Mississippi Valley, the hard red winter wheats have not measured up to the high standards of quality of grain which are characteristic of the better spring wheats. Marquis is undoubtedly the chief spring-wheat variety which is responsible for this difference. It is natural, therefore, that there should be a demand for winter wheats of better quality and greater winter hardiness than those varieties now being grown in this area. This paper deals with the inheritance of these important characters in crosses between spring

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[2] Reference is made by number (italic) to "Literature cited," p. 235.

and winter wheats and with the production of improved strains of hard red winter wheats which will withstand the severe winters of the North-Central States.

PREVIOUS STUDIES OF WINTER AND SPRING WHEAT TYPES AND INHERITANCE OF DIFFERENTIAL CHARACTERS

Linnaeus (*15*), in his "Species Plantarum," 1753, classified wheat as spring (*Triticum aestivum*) and winter (*T. hybernum*). Villars (*21*), in 1787, combined the two groups into *T. vulgare*. Clark, Martin, and Ball (*6*) classified varieties into the two classes, and other investigators (*8*, *10*) placed the wheats in three general groups according to their manner of growth, as follows:

(*a*) Winter-growing habit—those which, when sown in the spring, fail to head.
(*b*) Spring-growing habit—those which fail to survive the winter when sown in the fall in the more northerly regions but head normally from spring sowing.
(*c*) Intermediate forms—those which are hardy enough to survive the winter when fall sown and also mature as spring wheats when spring sown.

All fall-sown wheat varieties grown in the Great Plains area of the United States have the true winter habit. Most, if not all, of the wheats sown in the fall or winter in California and Arizona are true spring wheats.

Takahashi (*19*) classified barley varieties on the basis of growth habit and winter hardiness, as follows:

(*a*) Winter forms, which are winter hardy but fail to ripen when spring sown.
(*b*) Spring forms, which are killed when planted in the fall.
(*c*) Intermediate forms, which are winter hardy and also ripen when sown in the spring.
(*d*) Pseudowinter forms, which have the winter habit from spring sowing but are not winter hardy.

The first three groups in this classification of barley varieties correspond to those of wheat. The fourth class (pseudowinter) of the barleys undoubtedly comprises forms with the winter habit of growth which are not winter hardy. A similar class found in wheat is mentioned in this paper.

Fruwirth (*10*), Killer (*14*), Waldron (*22*), and Vavilov and Kouznetsov (*20*) demonstrated that spring types could not be selected from pure lines of winter wheats. The selection of spring types from winter wheats was successful only in those cases where both types were present before the selections were made. Fruwirth was not able to alter the growth habit of pure-line selections by growing these out of season for several generations. A single head of wheat was divided into two parts, and the seed from one-half was sown in the fall and that from the other half in the spring. The selections were grown from fall and spring sowing for eight years and then both lots were sown together in the two seasons. The growth habit and periods of blossoming and ripening of the two lots were found to be the same.

There are numerous reports in the literature on the relative resistance of wheat varieties to killing by low temperatures. There are relatively few reports, however, on the inheritance of cold resistance. Nilsson-Ehle (*16*), in Sweden, as early as 1900, began studies of the behavior of different varieties of winter wheat and their hybrids under various degrees of winter severity. Twelve years later he reported the results obtained from a cross between two medium-

hardy varieties of winter wheat. From this cross, lines were produced which extended beyond the limits of the parents in both directions. He concludes that the winter resistance character is inherited in the same manner as other quantitative characters and is controlled by several Mendelian factors.

Hayes and Garber (*13*), in Minnesota, report the production of winter wheats which were more resistant to injury by low temperatures than either of the parents. Two of these varieties, Minhardi and Minturki, were produced by crossing Turkey and Odessa. Odessa is a very winter-hardy variety which was obtained from Russia. Both Minturki and Minhardi have proved very winter hardy in Minnesota. They have proved even more winter hardy than the more hardy parent, Odessa, as has been demonstrated by Clark, Martin, and Parker (*7*), who have made a comparative test of the hardiness of winter-wheat varieties for a period of six years (1920–1925) over a wide area in the Northern States and adjacent Canada.

From crosses between two varieties of intermediate cold resistance, Åkerman (*3*), in Sweden, obtained hybrids, some of which were superior and others inferior to the parents. Most of the hybrids had an intermediate degree of cold resistance.

Schafer (*17*), in a brief report of inheritance studies at the Washington Agricultural Experiment Station, states that hardiness was recessive to nonhardiness in a cross between Turkey (winter) and Jenkin (club, spring) wheats. A majority of the F_3 rows showed severe winter injury.

Martin,[3] after a study of the inheritance of cold resistance in crosses between Kanred and Minhardi, concludes that it is difficult to study cold resistance without controlling temperatures. A large number of crosses of bulk F_3 generations were studied which were intermediate in winter hardiness as compared with their parents. Martin [4] and Vassar [5] both obtained a low correlation between the survival of winter-wheat hybrid lines in certain of the segregating generations and the progeny of individually selected plants from these lines in the following generation.

In 1918–19, 75 F_1 plants from a cross between Marquis and Kanred were grown in the plant-breeding nursery at St. Paul, Minn. The fact that 73 of these plants survived the winter would seem to indicate that hardiness of the winter parent was dominant or partially so. The later generations were all grown from spring seeding, and consequently no further studies of this character were made.

Detailed reviews have been published of the inheritance of growth habit in crosses between spring and winter cereals (*2*, *8*, *11*, *12*). Only a brief summary will be given here. Two investigators, Spillman (*18*) and Fruwirth (*9*), report a dominance of the winter over the spring habit of growth. Caporn (*5*) found the F_1 intermediate in time of ripening. Vavilov and Kouznetsov (*20*), Aamodt (*2*), and Gaines and Singleton (*12*) report a dominance of the spring over the winter habit of growth and conclude that the inheritance of this character is controlled by multiple factors. Cooper (*8*)

[3] MARTIN, J. H. COMPARATIVE STUDIES OF WINTER HARDINESS IN WHEAT. [In press.]
[4] MARTIN, J. H. Op. cit.
[5] VASSAR, L. P. FIELD AND LABORATORY METHODS FOR A DETERMINATION OF WINTER HARDINESS IN WINTER-WHEAT HYBRIDS. [Unpublished thesis, Univ. Minn.]

likewise reports a dominance of the spring over the winter habit, but explains his 13:3 F_2 ratio with a dominant factor for winter habit and an inhibiting factor of winter habit. The results reported by Cooper from the F_3 do not satisfactorily support the factorial hypothesis advanced for the F_2.

MATERIALS AND EXPERIMENTAL METHODS

The principal varieties of wheat used in this study are Marquis, Minhardi, and Minturki. Marquis, a high-yielding, high-quality, hard red spring wheat, was used as the spring parent in crosses with Minhardi and Minturki as the winter parents. Marquis is reported by Martin [6] as being one of the hardiest known spring wheats when sown in the fall. Cooper (*8*); at Ithaca, N. Y., found that the percentage of winterkilling in fall-sown Marquis was not great. At St. Paul, Minn., (*4*) fall-sown Marquis has not been known to live through the winter. In the experiments reported in this paper it was planted as a check in about every eleventh row throughout the nurseries.

The varieties used as the winter parents are two of the hardiest known winter wheats (*7*, *13*). Minhardi shows the highest average winter survival of all varieties grown in the extensive hardiness trials conducted by Clark, Martin, and Parker (*7*). Minturki in these same trials had the third highest percentage of survival. Both of these wheats are selections from a cross made at University Farm, St. Paul, Minn., in 1902, between Odessa (Minn. Accession No. 558) and Turkey (Minn. Accession No. 829) (*7*). Minturki, while not as winter hardy as Minhardi, is superior in yield, quality, and resistance to attacks of stem rust and is nearly the equal of Minhardi in cold resistance.

The plants were spaced 3 inches apart in the row and the rows were 1 foot apart. The F_1 plants were grown from both fall-sown and spring-sown seed. The F_2 from fall sowing were grown in two plots at some distance from each other, and killing was more severe in one location than in the other. In F_3 and F_4 in the winter-wheat nursery, each hybrid line for which seed was available was grown in four systematically distributed rows, each row containing 25 seeds. The parents were grown as checks in a similar manner about every eleventh row.

In order to average results from separate rows, a hardiness index figure was computed on the basis of 100 as representing a perfect stand of vigorous plants. The numbers of strong, weak, and dead plants were determined in the spring for each replication of each line. To obtain a figure for each row, the strong plants were given a value of 4, the weak 2, and the dead 0. The results were then calculated on a 25-plant basis, as there was not a full stand in all of the rows. The hardiness figures presented are averages of the hardiness of the separate trials.

In the F_2 the plants which had survived the winter were selected for growing in the F_3. Selections were made for early, medium, and late maturity. Others were selected on the basis of condition of the plant in the spring. Those that were large and vigorous in the spring were recorded as "strong," while those which barely survived were

[6] MARTIN, J. H. Op. cit.

recorded as "weak." In another experiment only vigorous plants were selected at random.

Numerous references and citations could be made regarding the physiological studies on the nature of cold resistance in wheat and many other plants. Martin [7] has recently made a comprehensive study of the literature and the methods employed to determine the hardiness of a given variety of winter wheat. He states that "most characters suggested for a test of winter hardiness, if dependable, hold for only a limited number of varieties or limited set of conditions." Studies of the inheritance of winter hardiness often are unsatisfactory because the winter may be too severe and may eliminate all or most all of the hybrid material, or it may be less severe and all of the plants may survive. The writers were fortunate in being so situated that the studies could be carried on under borderline conditions for killing. At University Farm, St. Paul, Minn., there is a rather consistent elimination of the less hardy plants and varieties and a survival of the hardier ones. In this study the determinations of winter hardiness were made in the field under conditions whereby the spring-wheat parent was eliminated when fall sown and the winter parent had a fairly high survival.

When seed was available, progeny of the same F_2 and F_3 plants which survived the previous winter were grown also as spring wheats. In the spring sowings the date of emergence of the main spike of each plant was noted and all of the plants were placed in groups by weekly heading periods. One week from the day on which the first plant headed, tags were placed on plants on which one or more spikes had emerged. These comprised the first class. One week later the plants which had headed after the first period were tagged. These comprised the second class. This process was followed until the end of the season, when no more plants headed. There were seven classes for heading periods and one class for nonheading, which comprised the true winter plants.

Following the selection of the plants in the field for vigorous growth, they were threshed individually and a determination of the seed quality was made in the laboratory. The seed of each plant was examined for hardness, texture, and color. Those which were hard, vitreous, and dark red were selected for growing in the following generation. If the seed of all of the plant selections from any line appeared to be low in quality, the seed of at least one plant of that line was sown, in order to study the inheritance of its characters in the following generation.

EXPERIMENTAL RESULTS

STUDIES OF F_1 AND F_2

The F_1 plants which furnished seed for the F_2 were grown in the spring of 1923. They headed approximately as early as the Marquis parent, while the winter-wheat parents, Minhardi and Minturki, when sown in the same spring, failed to head. The F_1 was also sown in the fall of the same year, in order to test its ability to survive the winter. F_2 generations, as well as the parental varieties, Marquis and Minhardi, were sown in the fall of 1923. None of the seed from

[7] MARTIN, J. H. Op. cit.

the F_1 plants was saved for spring sowing, as the primary purpose was to study the inheritance of cold resistance in relation to other characters which differentiated winter and spring wheats.

The results of these studies are given in Table 1. The winter was not particularly severe, and the Minhardi parent survived, 42 plants being classed as strong and 1 as weak. The F_1 plants were all winter-killed. It will be remembered that the F_1 headed practically as early as Marquis when sown in the spring. Apparently spring habit of growth is dominant over winter habit, and cold resistance behaves as a recessive character. From 33 to 55 per cent of the F_2 plants survived.

TABLE 1.—*A comparison of the survival of Marquis, Minhardi, and F_1 and F_2 generations of Marquis × Minhardi and Marquis × Minturki wheats at University Farm, St. Paul, Minn., in the winter of 1923–24*

Variety or cross	Total plants	Classified as—			Percentage of survival	Hardiness index
		Strong	Weak	Dead		
Marquis	100	0	0	100	0	0
Minhardi	43	42	1	0	100	99
Marquis × Minhardi, F_1	12	0	0	12	0	0
Marquis × Minturki, F_1	12	0	0	12	0	0
Marquis × Minhardi, F_2	978	429	105	444	55	49
Do	1,029	252	85	692	33	29
Total or average Marquis × Minhardi, F_2	2,007	681	190	1,136	43	39
Marquis × Minturki, F_2	1,138	453	152	533	53	49

HARDINESS IN F_3

Selection of individual plants was made in F_2, and the F_3 lines were grown in replicated short rows from fall seeding. When sufficient seed was available, the Marquis × Minhardi F_3 lines were also grown in individual rows from spring seeding. The parental varieties, Marquis, Minturki, and Minhardi, were grown throughout the fall-sown nursery at intervals of about every eleventh row and also were included in the spring-sown nursery. As has been stated, a selection of plants in F_2 was made in the early spring on the basis of strength of plant and at maturity on the basis of period of ripening. The data on winter hardiness of the various types of selections are given in Table 2.

TABLE 2.—*The relation of vigor and time of maturity in F_2 to hardiness in F_3 in hybrids from crosses of Marquis with Minhardi and Minturki wheats at University Farm, St. Paul, Minn.*

Variety or cross	Selection class in F_2	Number of lines	Number hardy as winter parent	Average hardiness index
Marquis		13		0
Minhardi		33		70
Minturki		15		67
Marquis × Minhardi, F_3	Strong	45	11	28
Do	Weak	45	0	11
Do	Early, good seed	35	2	27
Do	Early	20	2	17
Do	Midseason	20	2	34
Do	Late	20	0	20
Marquis × Minturki, F_3	Early	20	0	12
Do	Early, good seed	33	0	10
Do	Midseason	20	0	16
Do	Late	20	0	8

The winter of 1924–25 was more severe than that of the preceding year. Marquis was completely killed as usual, while Minhardi and Minturki had an average hardiness index of 70 and 67, respectively. Forty-five F_3 lines of Marquis × Minhardi, grown from seed of strong F_2 plants, had an average hardiness index of 28. Eleven of these were as hardy as the winter parent. Forty-five F_3 lines grown from seed of weak F_2 plants had an average hardiness index of 11, and none of these was as hardy as the winter parent. The selection of strong F_2 plants in the spring and the use of the progeny from these appeared to be worth while in this case. The selection of strong and weak plants was made in an F_2 row of Marquis × Minhardi in which winterkilling was especially severe. The selections for date of heading were made in plots of the F_2 of Marquis × Minhardi and Marquis × Minturki which were comparable, although winterkilling was less severe than in the plot where selections were made for vigor of plant. In the Marquis-Minhardi cross 55 plants which were selected for early maturity in the F_2 had an average hardiness index of 23.4 in the F_3; 20 plants selected for midseason maturity produced progeny with an average hardiness index of 34; and 20 plants selected for late maturity produced progeny with an average hardiness index of 20. The average hardiness index for all three groups was 24.8. Four lines from the early-maturing plants and two from those maturing at midseason were as hardy as the winter parent.

In the Marquis-Minturki cross, 53 plants were selected for early maturity in the F_2. The progeny of these 53 early plants had an average hardiness index in the F_3 of 10.7; 20 plants selected for midseason maturity produced progeny with an average hardiness index of 16; and 20 plants selected for late maturity produced progeny with an average hardiness index of 8. The average hardiness index for all three groups was 11.3. None of these lines was as hardy as the winter parent.

These results indicate that those plants selected for an intermediate heading period produced progeny with a higher hardiness index, on the average, than the progeny of parent plants selected for early or late heading.

The range of variability for the hardiness-index values in the F_3 can be appreciated by a comparison of the results obtained from the individual hybrid lines in relation to the parents (Table 3). There were 185 lines of crosses of Marquis × Minhardi and 93 from crosses of Marquis × Minturki. Thirty of the 185 lines of the cross of Marquis × Minhardi were within the range of variability for hardiness of the Minhardi parent, while 6 of the 93 F_3 lines of Marquis × Minturki were within the range of variability of Minturki. It is desirable to state again that these results are averages for four replicated rows and are therefore fairly reliable.

TABLE 3.—*Frequency distribution of parent checks and of 278 F_3 lines from crosses of Marquis with Minhardi and Minturki wheats at University Farm, St. Paul, Minn.*

Variety or cross	Class centers for hardiness index																		Number of lines	Average hardiness index
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	
Marquis	13																			13	0
Minhardi											4	2	2	6	4	3	6	5	1	33	70.3±1.4
Minturki									1			1	3	2	3	2	2	1		15	67.3±2.0
Marquis × Minhardi	26	23	23	20	19	18	14	3	4	5	9	7	7	4	1		1	1		185	22.4±1.0
Marquis × Minturki	29	21	13	11	5	4	3	1	1	2			3							93	11.1±1.0

Another method of comparison was used which helps to bring out the nature of the results. As the parents were grown in about every eleventh row it was possible to compare the F_3 lines directly with the nearby parents. If an F_3 line was as hardy as one of the nearby parent lines, it was considered practically as hardy as the parent. On this basis, 17 of the 185 F_3 lines of Marquis × Minhardi were as winter hardy as the parents, but none of the 93 F_3 lines of Marquis × Minturki were so winter hardy.

HARDINESS AND GROWTH HABIT IN F_3

The relationship between hardiness and habit of growth was studied for 105 Marquis-Minhardi F_3 lines, where sufficient seed was available, so that they were grown from both fall and spring sowings. The distribution of the F_3 lines of different growth habits in the hardiness-index classes is shown in Table 4. Five classes were used in distributing the F_3 lines for growth habit when spring sowing was practiced. These are as follows:

1. Homozygous winter, where all plants failed to head when sown in the spring.
2. Heterozygous winter, where segregation occurred with a predominance of the winter type and late heading.
3. Heterozygous intermediate, where segregation occurred with rather even distribution over all periods of heading.
4. Heterozygous spring, where segregation occurred with a predominance of the spring type.
5. Homozygous spring, where all plants headed as spring wheats.

TABLE 4.—*Correlation of growth habit and winter hardiness of 105 F_3 lines from crosses between Marquis and Minhardi wheats at University Farm, St. Paul, Minn.*

Growth habit	Class centers for hardiness index																				Number of lines	Average hardiness index
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90			
Marquis	13																				13	0
Minhardi											4	2	2	6	4	3	6	5	1		33	70.3±1.4
Homozygous, winter		2			1	2	1	1	2	1	5	3	6	2			1				27	46.5±2.3
Heterozygous, winter					3	2	1			2		1									9	31.7±2.8
Heterozygous, intermediate		8	9	7	7	5	3														39	15.1±0.8
Heterozygous, spring	3	6	4	1	2	1	2														19	11.1±1.4
Homozygous, spring	6	3		2																	11	4.1±1.4

This study shows that a correlation does exist between ability to survive the winter and winter habit of growth. Those lines which are homozygous for the winter growth habit have an average hardiness index of 46.5 ± 2.3. Those homozygous for spring habit of growth have an average hardiness index of 4.1 ± 1.4. The F_3 heterozygous lines for growth habit were divided into three groups. Those with a predominance of winter types had an average hardiness index of 31.7 ± 2.8; those which were like the F_2 distribution had an average hardiness index of 15.1 ± 0.8; and those in which spring types predominated had an average hardiness index of 11.1 ± 1.4.

While these results do show a general relationship between resistance to winterkilling and winter habit of growth, it is evident that some forms with a low winter resistance have the winter-growth habit, as some lines which were homozygous for winter-growth habit

killed out nearly as completely as the Marquis parent, when fall sown. Naturally, such strains are lost, because of their failure to head when spring sown, and their failure to survive when fall sown. Two of the lines when sown in the spring headed normally as late spring wheat and when sown in the fall had a fairly high hardiness index.

HARDINESS IN F_4

Selection was made in F_3 on the basis of hardiness index and seed quality. As a rule, unless the seed type was particularly promising, only a single plant selection was made in each of the F_3 lines which were low in winter hardiness. In those lines, however, which were as winter hardy as the winter parent in F_3, or approached this condition, larger numbers of plants were selected. Each F_4 line was the progeny of a plant selected in F_3, and, as in previous generations, was grown in the fall-sown nursery in four replicated rows, each containing 25 seeds. Nearly all F_4 lines were grown also as spring-sown wheats.

The frequency distribution for hardiness of the parents and crosses is given in Table 5. The severity of the winter was not greater than in 1924–25 when the F_3 was grown. The hardiness index of F_4 was much greater than that of F_3. This is without doubt a result of selection in F_3.

TABLE 5.—*Frequency distribution for winter hardiness of parent checks and of 533 F_4 lines from crosses of Marquis with Minhardi and Minturki wheats at University Farm, St. Paul, Minn.*

Variety or cross	Class centers for hardiness index																		Number of lines	Average hardiness index
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	
Marquis	53																			53	0
Minhardi										1	4		7	9	6	6	2	2		37	66.2±1.1
Minturki													1	4	1	3	4	2	1	16	74.7±1.4
Marquis×Minhardi, F_4		4	2	9	10	21	24	51	53	48	51	38	24	22	5	8	1			371	43.7±0.5
Marquis×Minturki, F_4	1	2	3	7	6	11	14	27	24	12	13	16	10	10	2	2	1	1		162	41.0±0.8

To determine the extent to which hardiness in F_3 is correlated with that in F_4, the hardiness index of the F_3 lines from which individual plants were selected was correlated with the hardiness index of progenies of these lines in F_4. The results are presented in Table 6. The correlation coefficient was $+0.1917 \pm 0.0282$. While this is mathematically significant in the light of its probable error, it is very low. This low correlation is in agreement with those obtained by both Martin[8] and Vassar.[9] A high correlation could not be expected for the reason that hardiness is a complex character and without doubt is dependent upon several genetic factors. In the heterozygous lines, plants with low cold resistance will be eliminated, and the occasional plant which survives in such heterozygous lines may be one which contains genetic factors for hardiness which are much above the average of the line.

[8] MARTIN, J. H. Op. cit.
[9] VASSAR, L. P. Op. cit.

TABLE 6.—*Correlation of winter hardiness in 1925 and 1926 in crosses of Marquis with Minhardi and Minturki wheats at University Farm, St. Paul, Minn.*

1925 winter hardiness	1926 winter hardiness																			
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	Total
0	1			4	1	1	4	1	2		1	1	1	2	1					20
5		3		1	1	4	4	6	5	4	5	4	1	4	1		1			44
10		1	1	2	3	2	5	1	4	3	6	4	2	2		1				37
15		2	1	2	2	5	1	4	6	4	5	5	4	4	2					47
20				1	1	5	4	6	6	4	4	5	2	2	1					41
25				1	1	1	5	6	3	4	5	2	4	1		2		1		36
30			1	2	4	8	3	9	12	5	5	5	3							57
35						1	1	4	3			2								11
40			1		1			6	5	8	2	1	1	1	2					28
45						1	1	4	2	2		3	1	2						16
50						1	1	9	8	6	6		2	3		1				37
55				1			3	7	6	6	8	6	2	1		1				41
60			1		2	1	4	11	8	4	11	12	7	8		1	1			71
65				2		2	2	2	5	7	5	3	2			1				31
70								1	1	1			1							4
75																				0
80																				0
85									1	2		1	1	2		2				9
90																				0
Total	1	6	5	16	16	32	38	77	77	60	63	54	34	32	7	9	2	1		530

$r=0.1917\pm0.0282$

As previously stated, nearly all F_4 lines which were grown as winter wheats were sown also in the spring. This was for the purpose of correlating the habit of growth with the winter hardiness. The F_4 lines were placed in five classes for growth habit, as in F_3. The classes were as follows: Those homozygous for winter habit, in which no plants headed when spring sown; those homozygous for spring habit, in which all plants headed when sowing was made in the spring; and three classes of heterozygotes, in which some plants headed and others failed to head. These three classes were made on the basis of the relative numbers of spring and winter types. It will be recalled that in F_3 there was a close relation between winter hardiness and habit of growth. No such close relation is evident in F_4, although a slight tendency for such a relation is apparent (Table 7).

TABLE 7.—*Correlation of growth habit and winter hardiness in F_4 lines from crosses of Marquis with Minhardi and Minturki wheats at University Farm, St. Paul, Minn.*

Variety or cross and growth habit	Classes for hardiness index																			Number of lines	Average hardiness index
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90		
Marquis, spring	53																			53	0
Minhardi, winter											1	4	7	9	6	6	2	2		37	66.2±1.1
Minturki, winter													1	4	1	3	4	2	1	16	74.7±1.4
Marquis × Minhardi, F_4:																					
Homozygous, winter		1	1	4	4	13	19	47	46	43	42	31	19	19	3	8	1			301	45.0±0.5
Heterozygous, winter				1	3	3		1	1	2	3		1	1						16	36.9±2.6
Heterozygous, intermediate		1	1	1	1	1	2	2	1	1	4	3	1		1					20	39.3±2.6
Heterozygous, spring		2		2	2	2	2	1	2	1	2	3	2		1					22	36.6±2.7
Homozygous, spring				1		2	1		1	1		1	1	1						9	40.0±3.7
Total		4	2	9	10	21	24	51	51	48	51	38	24	21	5	8	1			368	43.7±0.5
Marquis × Minturki, F_4:																					
Homozygous, winter		1	3	6	6	9	12	22	23	11	11	13	10	8	2	2	1	1		141	41.5±0.9
Heterozygous, winter		1				2	1	2	1		2	2		1						12	39.2±3.1
Heterozygous, intermediate				1			1	2												4	28.8±2.7
Heterozygous, spring																				0	
Homozygous, spring	1								1		1									3	26.7±7.5
Total	1	2	3	7	6	11	14	27	24	12	13	15	10	9	2	2	1	1		160	40.8±0.8

These data prove that correlation between growth habit and cold resistance is not complete. Apparently it is possible to obtain spring wheats which are as winter hardy as the most winter-resistant varieties. The heading period and winter-hardiness index of several of the hardiest of these types, which were homozygous for heading when spring sown, are given in Table 8. The heading periods contain all plants which headed during certain intervals of seven days. Nearly all of the Marquis plants headed in the first week. The hybrids headed considerably later. Marquis × Minhardi F_4 line 129–1 was spring sown in both F_3 and F_4. More than 100 plants were grown in F_3, and all headed during the second to the fourth week. There seems to be no doubt that this line is homozygous for ability to head when spring sown.

TABLE 8.—*Period of heading of individual plants of F_4 wheat hybrids of very winter-hardy lines in which all plants headed when sown in the spring at University Farm, St. Paul, Minn.*

Parent or cross	Hardiness index	Weekly heading periods			
		1	2	3	4
Marquis	0	56			
Marquis × Minturki, 264–3	47		18	1	
Marquis × Minhardi, 854–1	57	1	5	3	4
Marquis × Minhardi, 855–1	42	1	14		6
Marquis		147		4	
Marquis × Minhardi, 129–1	65		13	1	2
Marquis × Minhardi, 977–1	47		15	5	

DISCUSSION

A study of the interrelation of habit of growth and winter hardiness in crosses between spring and winter wheats has been made. In these studies winter hardiness is without doubt a result of cold resistance. The winter habit of growth was differentiated from the spring habit on the basis of ability to head when spring sowing was practiced. There was a close correlation between growth habit and cold resistance, although the linkage relation was not absolute. Some lines were obtained from these crosses which were homozygous for ability to head when spring sown and were also highly winter hardy as determined from actual test. These lines are similar to those of certain German investigators who have observed types which are winter hardy and also produce heads when sown in the spring. These lines probably originated from crosses of spring and winter wheats as a result of the recombination of genetic factors for cold resistance and for habit of growth.

From these results it appears that cold resistance is inherited in the same manner as other quantitative characters. A determination of the number or nature of factors involved in an expression of growth habit and cold resistance is not possible from these studies, as approximately 50 per cent of the plants were winterkilled in F_2, and it is impossible to say whether these types which were eliminated were of winter or spring habit. Without doubt the major part were of spring habit, as is shown by the close correlation in F_3 between spring habit and low winter resistance.

In the F_3 generation, 188 lines, each the progeny of an F_2 plant from the Marquis × Minhardi cross, were fall sown. In cases where all plants were not killed, at least one line from each F_3 was grown also in F_4. Four lines bred true in F_3 and F_4 for a hardiness index equal to that of the winter-hardy parent. This indicates that in these crosses several genetic factors are necessary to explain the inheritance of cold resistance.

SUMMARY

The inheritance of growth habit and cold resistance has been studied in generations of F_1 to F_4 crosses of Marquis with Minturki and Minhardi wheats. Winter wheats, when spring sown, failed to head, as a rule, at University Farm, St. Paul, Minn., while spring wheats such as Marquis were entirely winter killed, as a rule, when fall sown. The winter wheats Minturki and Minhardi are two of the most winter-hardy varieties for those sections where cold is a limiting factor.

The F_1 crosses headed as early as Marquis when sown in the spring and were entirely winterkilled when sown in the fall. Apparently spring-growth habit and low cold resistance are dominant over winter-growth habit and high cold resistance.

The F_2 generations were sown in the fall, each cross being grown in two replicated plots. The hardiness index values ranged from 29 to 49 for the four plots, and hardiness index values 0 and 99 were obtained, respectively, for Marquis and Minhardi, where 100 represented perfect hardiness. Selection was made in the spring for vigor of plant in the Marquis × Minhardi cross. Those plants that came through the winter without injury were called "strong" and those alive but seriously injured were designated "weak." Selection was also made at maturity, three classes being selected, early, midseason, and late.

F_3 and F_4 hybrids were each fall sown in four replicated single rows each containing 25 seeds. An average hardiness index on the basis of a perfect stand of 25 plants was computed by multiplying each strong plant by four, each weak plant by two, and each dead plant by zero, and placing the results on a 25-plant basis. When seed was available, the F_3 lines of Marquis × Minhardi were sown also in the spring. Rows of the parents were sown about every eleventh row throughout both nurseries.

The progeny of strong F_2 plants was, on the average, superior in winter hardiness to the progeny from weak ones. In both crosses the progenies of plants selected for midseason maturity were more winter hardy, on an average, than the progeny of those selected for either early or late maturity.

In F_3 there was a close correlation between habit of growth and winter hardiness in the lines of Marquis × Minhardi which were sown both in the fall and in the spring. Some lines which were homozygous for winter habit were winterkilled almost completely, and some lines, homozygous for heading when spring sown, were rather winter hardy.

Nearly all F_4 lines were sown both in the fall and in the spring. In the F_4 lines the differences in winter hardiness between the lines homozygous for winter or for spring habit of growth were not very great.

Cold resistance and winter habit were strongly correlated in inheritance, but the correlation was not absolute. Cold-resistant types which are capable of heading when spring sown probably are the result of a recombination of genetic factors for spring habit of growth obtained from Marquis with factors for cold resistance obtained from the winter-wheat parents, Minhardi and Minturki.

When the heading periods of lines of similar dates of sowing were compared, the hybrid lines which were homozygous for heading when spring sown, and which were highly cold resistant, matured later in all cases than Marquis.

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THE DEFERRED SHORT-TIME TEST AS A MEASURE OF THE PERFORMANCE OF DAIRY COWS[1]

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INTRODUCTION

In the official testing of dairy cows for production two types of test are commonly distinguished—the short-time test and the long-time test. The most common short-time test is for 7 days; the most common long-time test covers 12 months, or of late years, 10 months. At the present time the Holstein breeders are the only ones who make use of the short-time test. They have used it continuously and extensively under the present form of official supervision for the past 33 years (since 1894).

The long-continued and extensive use of the seven-day test may be taken as prima facie evidence that it possesses some merit. It has, nevertheless, some well-known faults. In the case of the more recent seven-day Holstein records the fat percentage of the milk frequently is too high to represent the usual performance of the Holstein cow. This defect of the short-time test was pointed out several years ago by Eckles (*4*),[2] who based his conclusions on experimental and observational evidence. Eckles concluded that "tests of dairy cows made for short intervals in the beginning of the lactation period can not be depended upon to indicate the normal per cent of fat produced by the cows tested."

Another charge often made against the short-time test is that the production record does not depend upon and does not measure persistency of lactation. Practically, it is the year's performance of a cow that is of importance from the standpoint of economical milk production; obviously, two cows with equal seven-day records might and often do have widely different records for the year. The relation between the seven-day records and the year records of Holstein cows has been fully treated by Yapp (*17*) and by Gowen and Gowen (*11*).

The criticisms of the seven-day test mentioned above apply to the test as usually conducted at an early stage of lactation. It is the purpose of the present paper to examine the possibilities of the *deferred* short-time test as affording a representative record of the performance of the cow.

Certain Holstein and Guernsey records are used in this examination. Guernsey breeders have never used the seven-day test (except for six records), but the yearly records of the breed were for many years published by calendar months and they are thus usable in connection with the present problem. It is proposed to examine these records with reference particularly to the evidence which they afford as to the possibility of eliminating the objectionable features of the short-time test by deferring its application to a comparatively late stage

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[2] Reference is made by number (*italic*) to "Literature cited," p. 249.

of lactation. Naturally, statistical methods have to be employed. The numerical values used from the records are those of fat percentage, milk yield, and, derived from these two, energy yield.

Energy yield is a very direct and exact measure of the work performed by the cow (*5*, *6*, *9*) and may be regarded as biologically the best measure of total production. It is estimated in terms of 4 per cent milk, designated as "fat-corrected milk" (F. C. M.), by the formula (9) F. C. M. $= 0.4M + 15F = M(0.4 + 0.15f)$ where M is milk yield, F is fat yield, using the same unit of weight throughout, and f is fat percentage. One pound F. C. M. = 1 pound 4 per cent milk = 340 calories (*2*, *13*, *14*).

Energy yield is a measure of the *amount* of work performed and fat percentage is a measure of the *direction* in which the work is performed. For economic reasons it is sometimes advantageous to produce milk fat with a minimum production of other milk solids (milk with high fat percentage), or vice versa (milk with low fat percentage). Energy yield and fat percentage are entirely independent variables and furnish the two most essential measures of performance.[3]

We may consider first the relation between the short-time test and persistency, approaching the relation through theoretical or generalized considerations.

LACTATION CURVE THEORY

By lactation curve is meant the curve describing the *rate* of milk secretion (rate of energy yield in the present connection) throughout the lactation period, or the portion of that period under consideration. The short-time test may be regarded as determining a point in the lactation curve. For example, the production for seven days may be taken as a measure of the *rate* of production per week at the middle of that week, if it is assumed that the lactation curve is linear.

The l near lactation curve may be expressed by the equation:

$$\frac{dy}{dt} = b - ct \qquad (1)$$

where y is yield in pounds of F. C. M., t is time in months, b and c are constants. Curves of this type are presented in Figure 1, A. The total yield for 12 months seems to be accepted almost by common consent as a highly valuable measure of production in official test practice. Figure 1, A, is constructed to represent the 12 months' record. Obviously, from the way the figure is constructed, the area under any one of the curves between the 0 and 12 ordinates represents the year's yield for that particular curve. It is

[3] The biological significance of fat percentage should be held clearly in mind. It is to be regarded as an expression of the ratio (×100) of the rate of fat secretion to the rate of milk secretion. Many investigators seem to have the point of view that fat yield is the result of milk yield and fat percentage. A more logical point of view seems to be that fat yield is the reusit of the rate of fat secretion, and that milk yield is the result of the rate of milk secretion, while fat percentage is the result of the relative values of these two rates. Fat percentage has a wider significance as a measure of performance because of its close correlation with protein percentage and energy value per unit milk.

The significance of energy yield is obvious, If we regard the cow as a machine, the *rate* of energy yield may be regarded as the *horsepower* delivered by the machine. A cow milking at the rate of 50 pounds F. C. M. per day produces the equivalent of a continuous delivery of 1.103 horsepower (1 calorie = 3,084 foot-pounds). A 1,200-pound horse at steady work may deliver about 0.9 horsepower for 10 hours per day. This is equal to a continuous delivery of 0.375 horsepower. An equivalent horsepower (i. e., 0.375 horsepower) is delivered by a cow milking 17 pounds F. C. M. per day, or 6,205 pounds F. C. M. per year.

also clear from mere inspection that a series of curves of different slopes but passing through a common point in the ordinate at $t=6$ will each produce a figure of the same area.

If the rate of yield per month at $t=6$ is determined, the area or year's yield is likewise determined regardless of the slope of the curve (provided, of course, it does not cut the base line). Hence, as far as persistency of lactation is concerned, there is apparently a possibility that a six-months-after-calving short-time test may possess as much merit as the year test.

That the areas under the three curves of Figure 1, A, are equal may be shown by the use of geometrical theorems—the equality of

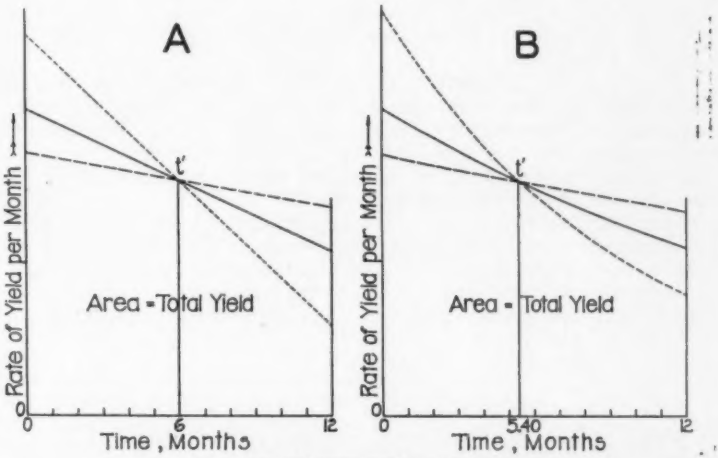

FIG. 1—Linear and exponential lactation curves

A, linear lactation curves. The area from $t=0$ to $t=12$ (year's yield) is completely independent of the rate of decrease in the rate of yield (persistency) at any fixed point (t') in the lactation curve and in the ordinate at $t=6$. That is, if the rate of yield at t' is determined, the year's yield is likewise determined.

B, exponential lactation curves. The area from $t=0$ to $t=12$ (year's yield) is practically independent of the rate of decrease in the rate of yield (persistency) at any fixed point (t') in the lactation curve and in the ordinate at $t=5.40$. That is, if the rate of yield at t' is determined, the year's yield is approximately determined.

NOTE.—The ordinates t' in A and B are the same height, although the one in B appears taller. This appearance is an optical illusion associated with the slopes of the curves. The height, apparent or real, of the t' ordinate is of no concern in the relationship which the figures illustrate.

opposite angles, etc. A much more elegant and comprehensive method is by way of the differential calculus, which serves to discover the ordinate in question as well as to demonstrate the property of equal areas under the curves. Integrating equation (1) we get: $y=bt-0.5ct^2+C$. Since $y=0$ when $t=0$, it is found by substitution that $C=0$ and hence,

$$y=bt-0.5ct^2.$$

Let $Y=12$ months' yield, $=12b-72c$.

Let $t'=$point in curve where Y is unaffected by variation in c.

Let $b'=$rate of yield at t', $=b-ct'$.

Then,

$$b = b' + ct'$$

and

$$Y = 12b' + 12ct' - 72c \quad \text{.....(2)}$$

It is necessary now to determine the value of t' at which a change in c produces no change in Y. Evidently this may be obtained by differentiating Y with respect to c in equation (2), setting the first derivative equal to zero, and solving for t', which gives

$$\frac{dY}{dc} = 12t' - 72 = 0$$

and

$$t' = 6.$$

That is to say, a point in the curve of equation (1) at $t = 6$ fully determines the area under the curve between the ordinates 0 and 12, regardless of the value of c, or the slope of the curve. When the area is interpreted in terms of milk yield it is necessary to impose the limitation that $b - 12c$ is not negative, since a negative rate of yield does not enter into the present problem.

It appears probable that the lactation curve is better represented as somewhat curvilinear rather than linear. The exponential equation introduced in connection with the lactation curve by Brody et al. (*3*), satisfies the requirements in this respect very well. In Figure 1, B, are presented lactation curves of the exponential type,

$$\frac{dy}{dt} = ae^{-kt} \quad \text{.....(3)}$$

in which y and t have the same meaning as before, a and k are the limiting constants of the particular lactation curve, and e is the base of natural logarithms. In equation (3), k is the factor that is affected by persistency and it is necessary to determine the point in the curve of the equation where the year's yield is unaffected by variation in persistency, or k. This may be done by a method similar to that just followed.

Integrating equation (3) gives $y = -\frac{a}{k} e^{-kt} + C$. Since $y = 0$ when $t = 0$, then $C = \frac{a}{k}$, and hence,

$$y = \frac{a}{k}(1 - e^{-kt}).$$

Let $Y = 12$ months' yield, $= \frac{a}{k}(1 - e^{-12k})$.

Let t' = point in curve where Y is unaffected by variation in k.

Let a' = rate of yield at t', $= ae^{-kt'}$.

Then

$$a = a' e^{kt'}$$

and

$$Y = \frac{a' e^{kt'}}{k} (1 - e^{-12k}) \quad \text{..... (4)}$$

Differentiating Y with respect to k in equation (4) and setting the first derivative equal to zero, gives

$$\frac{dY}{dk} = \frac{a' e^{kt'}}{k} 12e^{-12k} + (1 - e^{-12k}) \frac{ka't' e^{kt'} - a' e^{kt'}}{k^2}.$$

$$= Y\left[\frac{12e^{-12k}}{1 - e^{-12k}} + t' - \frac{1}{k}\right] = 0.$$

and

$$t' = \frac{1}{k} - \frac{12e^{-12k}}{1 - e^{-12k}}.$$

The value of t' varies with k, so that there is no fixed value as in equation (1). One recourse is to take a mean value for k. For cows on official test it appears (*3*, *7*, *10*) that $k = 0.05$ approximately. When $k = 0.05$, $t' = 5.40$. The indication is, therefore, that the short-time test should be deferred to about 5.4 months after the start of the year's record in order to most accurately indicate the year's record.

It is evident to anyone familiar with the course of milk secretion with advance in lactation that such a deferred short-time production is subject to many disturbing factors. This is true also of the year's yield. In the Guernsey records [4] the correlation between daily energy yield of the sixth full calendar month of the lactation and energy yield for the year has been computed (*7*) and found to be $r = 0.928 \pm 0.002$. There is little doubt that under similar environmental conditions a seven-day test conducted during the fifth month of the year-period would prove to be fully as highly correlated with the year's record as indicated by the above coefficient.

It seems, therefore, that the objection to the short-time test (as compared with the year test) namely, that it does not depend [5] upon persistency of lactation, may be disposed of by deferring the test to the fifth month of the lactation.

It has been assumed thus far that the year test is the ideal. But if we may judge from the practice of the milk producer, rather than the Advanced Register breeder, it is desirable to so manage the cow that she will bear a calf about every 12 months and have a dry period of about six weeks during each calving interval. There is other evidence (*8*, *15*) that such practice is consistent with the most economical production of milk. From Figure 2, it appears that in ordinary dairy practice a short-time test will afford the best index of production if it is conducted during the fourth month of lactation.

[4] All 365-day original entry records of volumes 33, 34, and No. 1 volume 35 of the Guernsey Herd Register (*1*) starting within 60 days after calving and in which conception did not recur within six months after calving.

[5] The year's record *depends* in part upon persistency but the year's yield of itself is a poor *measure* of persistency. The correlation between the year's yield and persistency [k of equation (3)] is $r = -0.226 \pm 0.016$. That the actual persistency value may be estimated with greater accuracy from an early seven-day test than from the bare figure of the year's yield is shown by the correlation between the initial rate of yield and persistency [a and k of equation (3)] $r = 0.535 \pm 0.012$.

The conclusions derived from Figure 2 are based on a calving interval of 12 months. In commercial milk production practice the calving interval naturally varies; but, accepting 12 months as the best length of the interval, the fourth-month test is still justified, even though the calving interval is shorter or longer than a year. From a biological standpoint, what is needed is a measure of pro-

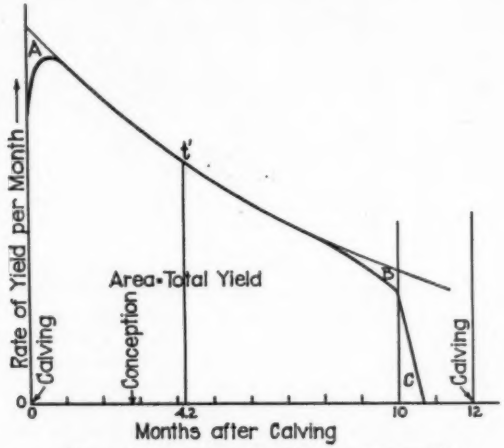

FIG. 2.—Ordinary average lactation curve (diagramatic)

The upper (lighter line) curve is the curve of equation (3) plotted for a value of $k=0.1$, since it is found under common practice that the average rate of decrease in milk yield with advance in lactation is around 9 to 10 per cent per month. The lower (heavier line) curve is to represent the realized lactation curve. For the most part, it is coincident with the curve of equation (3). Immediately following calving there is a deficiency in the rate of yield required by the equation which results in the area A. This is perhaps due to a residual inhibition from the preceding pregnancy. In the late pregnancy following there is again an appreciable deficiency in the rate of yield which results in the area B. This is probably due to an inhibition associated with the current pregnancy. Finally, starting at the ordinate 10, the cow is dried up in preparation for the next lactation. A yield represented by the area C is produced during this stage.

If it is assumed that A+B=C the lactation yield will be in accordance with equation (3) from $t=0$ to $t=10$. As an approximation equation (3) may be used between these time limits in determining the point (t') in the curve at which the area is independent of variability in k. By the method used above in the text it is found that $t'=\frac{1}{k}-\frac{10e^{-10k}}{1-e^{-10k}}$. For $k=0.1$, $t'=4.2$. A short-time record at this stage of lactation should of itself afford a valuable measure of production. The ratio of the lactation yield (that is, 10 months by the equation) to the rate of yield per month at $t=4.2$, is given by $\frac{\frac{a}{k}(1-e^{-10k})}{ae^{-4.2k}}$. Where $k=0.1$ this ratio=9.62. The corresponding ratio to the rate of yield per week is 41.8. The ratio is only slightly affected by ordinary differences in k.

ductive capacity which is physiologically sound, even though it may be faulty from a cost-accounting standpoint. The cost accountant is interested in the average production per unit time over the calving interval. But the calving interval is dependent upon the recurrence of conception, which is independent of productive level (*8*). Reproductive capacity is a very important matter, but it is a separate problem from lactation capacity. The fourth-month test, therefore, holds its merit as a biological measure of the milk productive capacity of the cow.[6]

[6] It is quite possible that an early short-time test may be a better biological measure of lactation capacity than the deferred short-time test, or year test itself, if environmental conditions which influence the lactation curve are unfavorable or irregular. The ideal biological measure would seem to be one describing the initial value and slope of the lactation curve under standard environmental conditions.

As far as energy yield is concerned it seems from the theoretical and practical solutions given, that the deferred short-time test has much merit. Let us now examine the case with respect to fat percentage. Evidence here is available from both the Guernsey and Holstein published advanced registry records.

FAT PERCENTAGE OF GUERNSEY RECORDS

Both fat and milk yields of farrow Guernsey cows follow the curve of equation (3), although not as closely as does energy yield (*10*). The stage of the record at which the fat percentage at the time is the same as the fat percentage for the year may be computed theoretically from the fat and milk lactation curves, as follows. Let $a_1e^{-k_1t}$ represent the rate of fat yield and $a_2e^{-k_2t}$ the rate of milk yield. Then the time (t) at which the fat percentage of a short-time test should be the same as the fat percentage for the year test is given by the value of t in the equation,

$$\frac{100a_1e^{-k_1t}}{a_2e^{-k_2t}} = \frac{100a_1\dfrac{1-e^{-12k_1}}{k_1}}{a_2\dfrac{1-e^{-12k_2}}{k_2}}$$

or

$$e^{(k_2-k_1)t} = \frac{k_2(1-e^{-12k_1})}{k_1(1-e^{-12k_2})}.$$

If the group values found (*10*) for k_1 and k_2, namely, $k_1 = 0.034202$ and $k_2 = 0.052347$, are taken, it is found that $t = 5.48$. This value is practically identical with the point of closest correlation between the short-time yield and the year's yield. Hence, it appears that as far as the customary year record is concerned a deferred short-time test conducted during the fifth month should be directly representative of the fat percentage.

On the basis of the actual records (above noted) the relation between the several monthly fat percentages and the yearly fat percentage may be examined. The records are considered in two groups according as the test was conducted under the one-day or two-day monthly supervision plan. Records from tests conducted partly under one and partly under the other plan are not used. The statistical constants derived from the records are given in Table 1, and these constants are graphically shown in Figure 3.

It may be noted that the mean fat percentage curve of Figure 3 shows a tendency to a convex upward form, whereas the fat percentage curve derived from the exponential lactation curves for fat and milk as given in Figure 1, B, is very slightly convex downward. This discrepancy is probably associated with the season of calving (*16, fig. 3*).

The chief item of interest is the correlation between the one-day or two-day fat percentage and the yearly fat percentage. The change in the coefficient of correlation with advancing lactation is fairly regular in the case of the two-day test (fig. 3), and if the curve is smoothed it is found that the highest correlation is reached at about five months after calving. The correlation indicated at this time is about 0.83. This indicates that a five-months' deferred test should give a fairly accurate value of the year's fat percentage.

FAT PERCENTAGE OF HOLSTEIN RECORDS

As previously stated, it is known that many seven-day records are misrepresentative of the *usual* performance of the cow, particularly with respect to the fat percentage. If the seven-day records from tests conducted at successively later stages of lactation are considered, it may be possible to find a stage where the records seem to be more representative. The question of a standard by which to judge the representativeness of the record arises. One possibility is to take as a standard the seven-day records made early in the history of advanced registry testing, before the practice of specially fitting the cow for the seven-day test had come into operation.

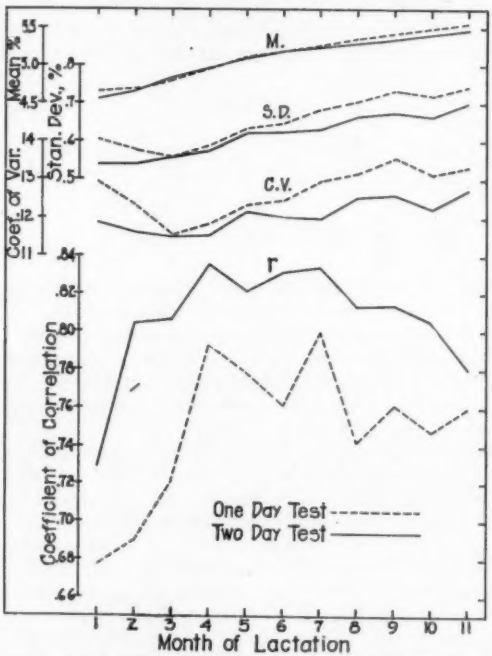

FIG. 3.—Constants of monthly fat percentages and correlation between monthly and yearly fat percentages—Guernsey records

The fat percentage and milk yield constants of certain early records (1894–1898) are given in Table 2. Table 2 gives also similar constants for certain later seven-day records (1919–1921) classified according to the stage of lactation. The mean fat percentage of the early records is 3.280 while that of the recent records is 3.485, including records started 6 to 99 days after calving. Records started at 6 to 9 days after calving have a mean of 3.756. The mean fat percentage continues to decrease up to 60 days after calving. Taking the early records as an index of normalcy, the fat percentage at this stage (60 days after calving) is quite normal. There is naturally some increase in fat percentage with advance in lactation (*16*).

TABLE 1.—*Statistical constants of monthly and yearly fat percentages for Guernsey records*[a]

Period of lactation	Mean		Standard deviation		Coefficient of variability[b]		Coefficient of correlation between monthly and yearly fat percentage	
	One-day test	Two-day test	One-day test	Two-day test	One-day test	Two-day test	One-day test	Two-day test
	Per cent	*Per cent*	*Per cent*	*Per cent*				
First month	4.660±0.025	4.550±0.012	0.603±0.018	0.539±0.008	12.95±0.38	11.84±0.18	0.677±0.022	0.728±0.010
Second month	4.684± .023	4.656± .011	.579± .016	.540± .007	12.36± .34	11.59± .16	.690± .020	.804± .007
Third month	4.804± .022	4.841± .011	.557± .015	.557± .008	11.59± .32	11.50± .16	.722± .019	.808± .007
Fourth month	4.969± .023	4.963± .011	.589± .016	.574± .008	11.85± .33	11.56± .16	.792± .015	.836± .006
Fifth month	5.120± .025	5.097± .012	.631± .017	.621± .009	12.32± .34	12.18± .17	.778± .015	.821± .006
Sixth month	5.190± .025	5.191± .012	.646± .018	.624± .009	12.45± .34	12.02± .16	.761± .016	.831± .006
Seventh month	5.277± .027	5.256± .012	.684± .019	.631± .009	12.96± .36	12.00± .17	.800± .014	.834± .006
Eighth month	5.360± .028	5.299± .013	.707± .019	.667± .009	13.19± .36	12.59± .17	.743± .017	.813± .007
Ninth month	5.416± .029	5.359± .013	.736± .020	.676± .009	13.58± .37	12.61± .17	.762± .016	.814± .007
Tenth month	5.481± .028	5.407± .013	.720± .020	.663± .009	13.13± .36	12.25± .17	.748± .017	.805± .007
Eleventh month	5.574± .029	5.494± .014	.744± .020	.702± .010	13.35± .36	12.77± .17	.761± .016	.780± .008
Year	5.069± .019	5.047± .010	.478± .013	.490± .007	9.44± .26	9.72± .13		

[a] The number of records taken for the different test periods were as follows: One-day test, first month, 267—subsequent months and year, 301; two-day test, first month, 1,000—subsequent months and year, 1,205.

[b] The probable errors of the coefficients of variability in Tables 1 and 2 are computed by the approximate formula $E_{cv}=100E\sigma/M$.

TABLE 2.—*Seven-day Holstein-Friesian records classified according to time after calving at which started*

Time after calving	Chart (fig. 4)	Number of records [a]	Fat percentage			Fat percentage [b]	Milk yield			Mean F. C. M.
			Mean	Standard deviation	Coefficient of variability		Mean	Standard deviation	Coefficient of variability	
							Pounds	*Pounds*		*Pounds*
	A	277	3.280±0.0166	0.410±0.0117	12.49±0.36	3.254	365.6±3.71	91.5±2.62	25.05±0.76	324.7
6 to 99 days	B	12,048	3.485± .0028	.449± .0020	12.87± .57	3.469	405.1± .53	85.8± .37	21.18± .91	372.8
6 to 9 days	C	2,106	3.756± .0060	.547± .0057	14.56± .15	3.734	391.9±1.16	78.7± .82	20.08± .21	376.2
10 to 19 days	D	3,451	3.525± .0050	.432± .0035	12.26± .10	3.507	412.8± .97	84.6± .69	20.49± .17	382.2
20 to 29 days	E	1,825	3.425± .0060	.378± .0042	11.04± .12	3.409	417.5±1.39	88.3± .98	21.15± .23	380.5
30 to 39 days	F	2,132	3.366± .0056	.386± .0040	11.47± .12	3.353	410.5±1.29	88.1± .91	21.46± .22	370.8
40 to 49 days	G	1,083	3.359± .0076	.372± .0054	11.09± .16	3.343	402.2±1.83	89.1±1.29	22.15± .32	362.6
50 to 59 days	H	565	3.330± .0108	.382± .0076	11.47± .23	3.319	395.2±2.45	86.2±1.73	21.81± .44	354.9
60 to 69 days	I	392	3.345± .0136	.398± .0096	11.91± .29	3.330	381.1±2.77	81.3±1.96	21.33± .51	342.8
70 to 99 days	J	494	3.366± .0103	.340± .0073	10.09± .22	3.352	374.0±2.39	78.8±1.69	21.08± .45	337.7
240 to 269 days	K	1,012	3.485± .0093	.438± .0066	12.56± .19	3.465	329.4±1.76	83.1±1.25	25.23± .38	302.9

[a] The records of chart A are not classified as to time after calving. They are original entries of the first 277 cows entered under the present system of official supervision, vols. 1–9 of the Holstein-Friesian Advanced Register (*12*). Charts C and D are original entries of vol. 31. Charts E–J are original entries of vols. 30–32. Charts C–J are combined in Chart B. This combination is not quite proper since part of the records came from one volume and part from three volumes. Chart K is derived from the "special records" of vols. 24–31 (*12*).

[b] Weighted by milk yield.

Another way in which the records may be judged is in connection with the relation between fat percentage and milk yield. It may be assumed that energy yield should be constant at various fat percentages if conditions are normal. The milk yield curve is accordingly $y = A/(2.66 + f)$, where f is fat percentage and A is a constant taken as the average energy yield (in units of 51 calories) for the entire group. That is, $A = \Sigma\, n M_o\, (2.66 + f)/\Sigma\, n$, where M_o is the observed milk yield at the given fat percentage, f, and n is the frequency.

In Figure 4, chart A, this curve is given for the early age-corrected records, A having the value 2.47.

The fat percentage frequency distribution of these early records is also given in chart A. The smooth frequency curve is merely a free-hand sketch.

Similar graphic presentation for the other seven-day records of Table 2 is given in Figure 4, charts B to K. The milk yields in these charts are not corrected for age and the values of A may be had from the respective values given in the last column of Table 2, by multiplying by 6.66. The smooth frequency curve is repeated from chart A as a basis of comparison.

By studying the charts of Figure 4, one may form an idea as to when and to what extent the seven-day records conducted at various stages of lactation approach representative values. The comparison is of course a rough one. Certain yearly records [7] are given in chart L as of interest.

Judging by the data of Table 2 and Figure 4 it would seem that deferring the seven-day test to 60 days or more after calving would eliminate the exceptional conditions and misrepresentative features of the seven-day test as conducted a few days after calving.

SUMMARY

Two objections have been raised to the record of the seven-day test conducted shortly after calving; (1) that the fat percentage obtained is not representative, and (2) that the record is not dependent on persistency of lactation. Data from the Guernsey and Holstein advanced registry are examined with respect to the possibility of eliminating these objections by deferring the test to a later stage of lactation. It appears that the objection on the score of the fat percentage may be overcome by deferring the test to 60 days or more after calving. The objection on the score of persistency may be overcome by deferring the test to the fifth month of lactation. To best represent the practically useful lactation capacity of the cow (in distinction to the maximum record of advanced-registry practice) the short-time test should be conducted during the fourth month of lactation.

[7] All long-time records, including reentries, from vols. 24–30, Holstein-Friesian Advanced Register (1912–1919) (*12*).

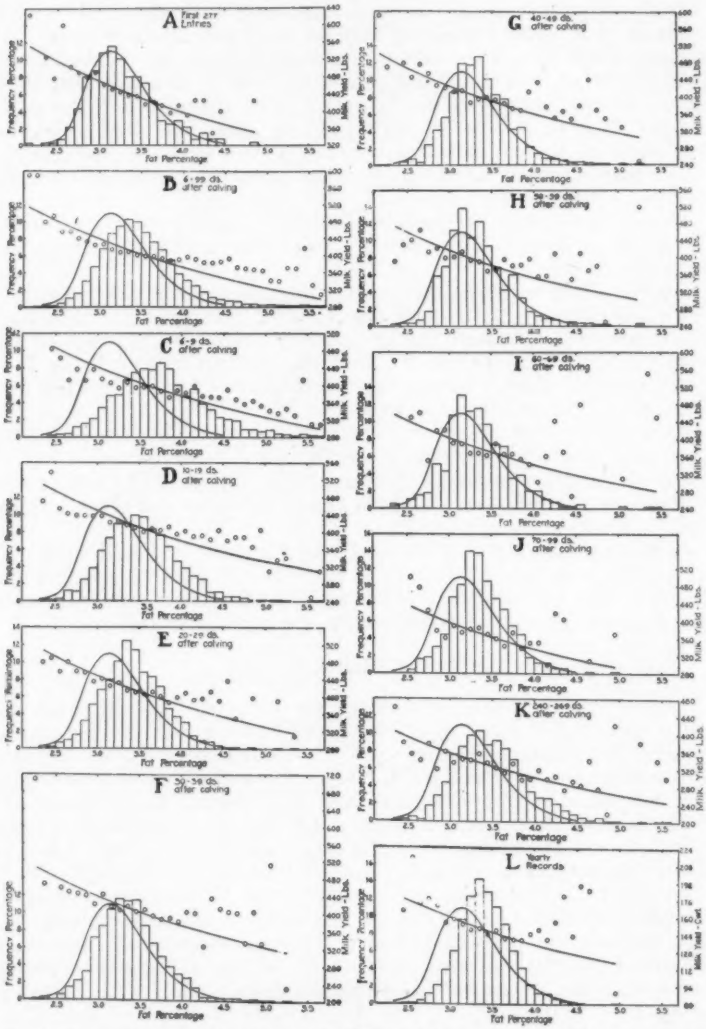
A First 277 Entries
B 6-99 ds. after calving
C 6-9 ds. after calving
D 10-19 ds. after calving
E 20-29 ds. after calving
F 30-39 ds. after calving
G 40-49 ds. after calving
H 50-59 ds. after calving
I 60-69 ds. after calving
J 70-99 ds. after calving
K 260-269 ds. after calving
L Yearly Records
Frequency Percentage
Milk Yield - Lbs.
Milk Yield - Cwt.
Fat Percentage

FIG. 4.—Frequencies (columns) and mean milk yields (circles) at various fat percentages for Holstein record

Chart A represents the seven-day records of the first 277 cows admitted (1894–1898) to the advanced register under the present system of official supervision. Charts B to K represent seven-day records classified according to the time after calving at which the record was started. Chart L represents yearly records. The free-hand frequency curve of chart A is repeated in charts B to L. The milk yields in chart A have been corrected for age, increasing the average yield to 122.43 per cent of the figure given in Table 2. The milk yields in charts B to L have not been corrected for age.

Observations at the following fat percentage classes are not shown in the charts indicated:

Classes	5.75	5.95	6.05	6.25	6.35	6.45	6.65	6.95
Charts	B, G	B, C	B, D	B, C	B, C	B, C	B, C	B, C

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PROTECTION AFFORDED THE SKIN AGAINST SUNBURN BY TEXTILE FIBERS[1]

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INTRODUCTION

Recent interest in the effect of ultra-violet rays on the human organism suggested a study to determine the protection from sunburn afforded the skin by textile fibers. No records could be found of any work of this nature having been done under controlled conditions.

Some experiments were performed by Hess[2] to determine the ability of certain fabrics, suitable for infants' clothing, to transmit ultra-violet rays. A carbon arc lamp was used as the source of radiation and rats were employed as subjects. The results were determined wholly by the biological effect of the rays on the animal organism. The application of the above research, however, to this problem of protection against sunburn is not direct.

Since work has been done on the ability of rayon, the artificial textile fiber, to transmit ultra-violet rays, it was not included in this work.

THE PROBLEM

The object of this investigation was to determine the relative ability of the textile fibers wool, silk, linen, and cotton, to screen out ultra-violet rays from direct sunlight and from artificial light sources. It was necessary to work with the fibers in the form of fabrics.

METHOD OF PROCEDURE

In choosing the materials with which to experiment an effort was made to obtain fabrics woven of wool, silk, linen, and cotton, of the same weight and containing the same number of threads per inch, in which case the spaces between the threads would have been the same. This was impossible and could be done only by having the fabrics woven according to definite requirements.

The problem was arranged so as to determine the ratio of the time required to sunburn the skin through certain fabrics to the time required to produce a sunburn of equal intensity upon the unprotected skin under the same controlled conditions. This ratio was accepted as the coefficient of protection of the fabric. The difference in protection by the fabrics not due to difference in construction was accepted as the coefficient of protection of the fiber in the fabric.

The lightest weight wool fabric, plain weave, was chosen and this was matched as nearly as possible with respect to weight and number

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[2] HESS, A. F., and WEINSTOCK, M. A STUDY OF LIGHT WAVES IN THEIR RELATION TO RICKETS. Jour. Amer. Med. Assoc. 80: 689. 1923.

of picks and ends per inch, with similar fabrics of silk, linen, and cotton. The problem of the absorption due to color was avoided by choosing only white materials. Plain weave, which contained rectangular spaces between the threads and thus permitted the measurement of these threads, was used.

A complete textile analysis of fabrics used in the initial work was made. The data secured in these analyses are reported in Tables 1, 2, 3, and 4. Table 1 includes the commercial names and descriptions of fabrics; Table 2 analyses of the yarns used in their construction; and Table 3 gives detailed facts concerning the fibers used in yarns. Column three in Table 4 shows the per cent of interspace or the ratio of the total area of the fabric to the interspace included.

TABLE 1.—*Description of fabrics used in experiment*

Name of fabric	Fiber	Width in inches	Price per yard	Weave	Color	Weight (4 by 4 inch piece)	Breaking strength		Finish
							Warp	Filling	
						Grams	*Pounds*	*Pounds*	
Nun's veiling	Wool	44	$1.95	Plain	White	1.000	18	9	Bleached soft.
Pongee	Wild silk.	36	2.25	do	do	.586	25	21	Bleached, slightly gummed.
Handkerchief	Linen	36	1.65	do	do	.534	12	8	Bleached, soft.
Muslin	Cotton	36	.17	do	do	.816	32	16	Starched, bleached.

TABLE 2.—*Description of yarn in fabrics used in experiment*

Fabric	Yarn, size	Number per inch		Width in inches		Breaking strength		Twist per inch		Ply, warp filling
		Warp	Filling	Warp	Filling	Warp	Filling	Warp	Filling	
						Ounces	*Ounces*			
Wool	54	70	65	0.01040	0.00950	8	6	11	6	Single.
Silk	16/18	73	72	.00797	.01007	10	9	4	0	Do.
Linen	28	94	72	.00587	.00562	10	9	16	25	Do.
Cotton	40	78	72	.00521	.00625	16	9	19	13	Do.

TABLE 3.—*Length, tensile strength, stretch, and quality of fibers in yarn*

Fabric	Number of fibers in yarn		Length of fibers	Tensile strength of fibers		Stretch of		Quality of fibers
	Warp	Filling	Warp filling	Warp	Filling	Warp	Filling	
				Grams	*Grams*	*Cm.*	*Cm.*	
Wool	40	25	0.5 to 3 inches	11.61	5.59	25	13.0	Very good.
Silk	25	25	Length of piece	5.85	5.52	13	12.6	Good.
Linen	30	25	0.5 to 3 inches	6.45	6.46	15	13.0	Medium.
Cotton	34	45	0.5 to 2 inches	5.59	4.30	13	10.0	Do.

It was decided to test the effect of exposure to direct sunlight and to the lamp on the same subjects. So far as the quality of burns produced was concerned no difference could be detected between the burn produced in direct sunlight and by the ultra-violet lamp.

TABLE 4.—*Comparison of space covered by the threads with the space between the threads in fabrics studied in experiment*

Fabric	Space covered by thread (square inches)	Interspace (square inches)	Ratio of the total area of the fabric to the interspace included
Wool	0.89820	0.10180	9.80
Silk	.9192	.0827	12.08
Linen	.81820	.18180	5.50
Cotton	.81140	.18860	5.30

It seemed especially desirable to devise a physical test that could be used as a means of indicating approximately the necessary time of exposure, thereby eliminating the necessity of so many preliminary exposures. With this in view it was suggested that possibly the ability of these materials to transmit visible light which could be tested by photometry, could be used in a measure as a test of their ability to transmit ultra-violet rays, since these rays are shorter than the rays in the visible part of the spectrum. Furthermore, since the interspace between threads is usually many times greater than the wave length of ultra-violet rays, it might follow, provided the thread transmitted no light, that the ratio of the area covered by the threads to the area of the interspace would be a measure of the protection afforded.

Tests made on photographic paper of the printing-out type indicated that the paper used was sensitive only to the ultra-violet rays, and that the ratio of the time required to produce a given tone on the paper when shielded by the fabric in question to the time required to produce the same color under the unscreened light might indicate the ratio sought.

Since measurements were to be made by means of comparing the color tone of the effect of light on sensitized paper and on the skin, it was very important that the operator be able to accurately match color. Tests were made by exposing one-half inch of the printing-out paper to the light for a few seconds, one-half inch more for an additional equivalent length of time, and so on, until two whole sheets of paper had been exposed, the first strip being thereby exposed for the full time, and the time for each succeeding strip being decreased by an equal number of seconds. This gave a graduation in tone from white to very dark brown. The strips were numbered on the back and the sheets were cut in strips, 20 in all. Since the operator was able to arrange these strips in order according to tone, her ability to match color within at least a 5 per cent error was established. All light-exposed paper was fixed without toning in hypo solution at 22° C., and rinsed in running water.

EXPERIMENTAL WORK

A number of persons were tested to determine their sensitiveness to sunburn, both in direct sunlight and under the lamp. The arm of the subject was covered with a brown woolen sleeve from which a small piece about 1-inch square had been cut. Tests were made with a 5-minute exposure to direct sunshine, during the month of

June, with no perceptible results. The time of exposure was increased until a good burn was obtained. In all the work done the time of exposure was regulated so that blistering the skin was prevented and the burns made were of intensity sufficient to result only in a deep reddening, or erythema, which was followed by peeling and a subsequent tanning. Four of the subjects tested burned with a 30-minute exposure; one required a 45-minute exposure to produce a burn of the same intensity; and one subject was found who showed no evidence of burning even after an exposure of one hour. This person was of medium-light complexion and perspired freely. Contrary to the opinion commonly held, it was not found that the fairest skin always burned in the shortest time.

It was found that approximately only one-tenth as much time was required to obtain a burn under the quartz mercury lamp with the skin 1 foot from the lamp and a voltage over the terminals of 70 to 80, as was required to produce a burn of the same intensity in direct sunlight, between the hours of 11 and 3 on a bright day, during the months of June, July, and August. An air-cooled quartz mercury arc lamp was used, and tests were made only after voltage became constant.

The screening effect of the fabrics to visible light was obtained by means of the photometer; and the intensity of the light transmitted by the fabric was checked with the candlepower of the unscreened light. The fabric was interposed directly in front of one of the openings in the photometer screen, being fastened to it by means of elastic bands. In this manner the following ratios were obtained:

Fabric	Protective ratio
Wool	7. 07 to 1
Silk	7. 11 to 1
Linen	4. 39 to 1
Cotton	5. 86 to 1

These figures show that the wool and silk fabric transmitted only one-seventh, the linen a little less than one-fourth, and the cotton more than one-fifth of the incident visible light.

The percentage of interspace in the fabric or the ratio of the space between the threads to the space covered by the threads was determined by means of a micrometer microscope. By this method only an approximate determination could be made. Ends of fibers protruding into the interspace tend to cut down the space, but the amount of this reduction could not be measured; untwisted yarns permit passage of light through them; and the uneven line produced by the twisted thread influences the area of the interspaces. Assuming that the light passed through the interspaces and only through the interspaces, the ratios of protection offered by these fabrics would be:

Fabric	Protective ratio
Wool	9. 8 to 1
Silk	12. 0 to 1
Linen	5. 5 to 1
Cotton	5. 3 to 1

From the above it is plain that about one-fifth of the light might pass through the interspace in the case of the cotton and linen fabrics, whereas with wool and silk the quantity was somewhat less. These tests show that the number of picks and ends per inch can not be taken as a measure of closeness of weave unless the size of the thread is also known. (See Table 2.)

As previously stated, the screening effect of these fabrics to ultra-violet light was determined by means of a printing-out paper. The color obtained by 15 seconds' exposure of the paper to the rays of the lamp was decided upon as the best color to match. With a 15-second period as a basis therefore, the fabrics were tested to determine the ratio of the time required to obtain the same color tone when the paper was screened with the fabric in question. Exposures were made 1 foot from the lamp with a relatively constant voltage of 70 to 80.

For this work it was found necessary to keep either the paper or the fabric moving slightly in order to secure a uniform color tone.

The first four fabrics tested as shown in Table 5 gave the following results:

Fabric	Protective ratio
Wool	8.5 to 1
Silk	13.0 to 1
Linen	3.0 to 1
Cotton	4.0 to 1

That is, it took 8.5 times as long, or 2 minutes and 7 seconds, to obtain the same effect on the paper used when screened by the wool fabric as was obtained in 15 seconds' exposure of the unprotected paper.

TABLE 5.—*Coefficient of protection of textile fabrics*

Fabric	Coefficient determined by—			
	Photometer	Measurement of interspace	Action of ultra-violet rays on sensitized paper	Action of sunlight on skin
Wool	7.07	9.80	8.50	20.00
Silk	7.11	12.00	13.00	20.00
Linen	4.39	5.50	3.00	3.00
Cotton	5.86	5.30	4.00	4.00

The above ratios were used as a basis for time of exposure of the skin to direct sunlight when protected by the different fabrics. By using the ratio obtained by the action of ultra-violet light on sensitized paper good burns of an intensity equal to the control were secured through cotton and linen.

Several unsuccessful attempts were made to secure burns through the silk and wool fabrics in direct sunlight. No burns were obtained of sufficient intensity to show discoloration for more than 48 hours, although the ratio of 6 to 1 used required 4 hours and 15 minutes exposure. A 45-minute exposure was necessary to secure a positive burn on the unprotected skin of the subject.

An unscreened control of correct time for the individual subject was run parallel with every fabric test. This permitted the direct comparison of results and eliminated errors which might be due to uncontrollable conditions, such as the quantity of ultra-violet rays in the source of light at the particular time of exposure, and the individual's resistance to the effect of these actinic rays. The subject upon whom the largest number of the tests was made varied in her sensitiveness to sunburn. Twice during the 18 months through

which the experiments were continued, this subject showed no signs of sunburn under double the normal time of exposure, and the same controlled conditions of distance and voltage.

The fabrics were next tested under the lamp, on the same subjects, as illustrated in Figure 1. It was found that the ratios used in direct sunlight tests gave the same results under the lamp with the time of exposure reduced to one-tenth. Thus it required an exposure of 1½ hours to direct sunlight to secure the same intensity of burn through

FIG. 1.—Method used in testing fabrics under the lamp

linen as was obtained in 9 minutes under the lamp. A burn that required 2 hours' exposure in direct sunlight was produced in 12 minutes by the rays of the lamp.

In tests of the silk fabric the ratio was increased to 13 to 1, the same ratio obtained by screening printing-out paper from the rays of the lamp, without perceivable results. It was found that a ratio of 20 to 1 was required to obtain a burn through this fabric.

The same method was used in testing the wool fabric, as illustrated in Figure 2, until by experiment it was found that it also required a ratio of 20 to 1.

SCREENING TESTS

Screening tests were made of these fabrics by the use of the spectrograph and the quartz mercury arc lamp. The fabric was fastened to a screen and interposed directly in front of the collimator, the prism having been set for minimum deviation. Under 5 seconds' exposure

for control and the ratios obtained by means of the lamp and the printing-out paper for time of exposure through the different fabrics, the ratio of 3 to 1 for linen was found to be sufficient to give a spectrograph (through the fabric) of as great a density as the unscreened control. The ratio of 4 to 1 was only slightly low for cotton. These ratios had proved correct for ratio of time of exposure to produce a

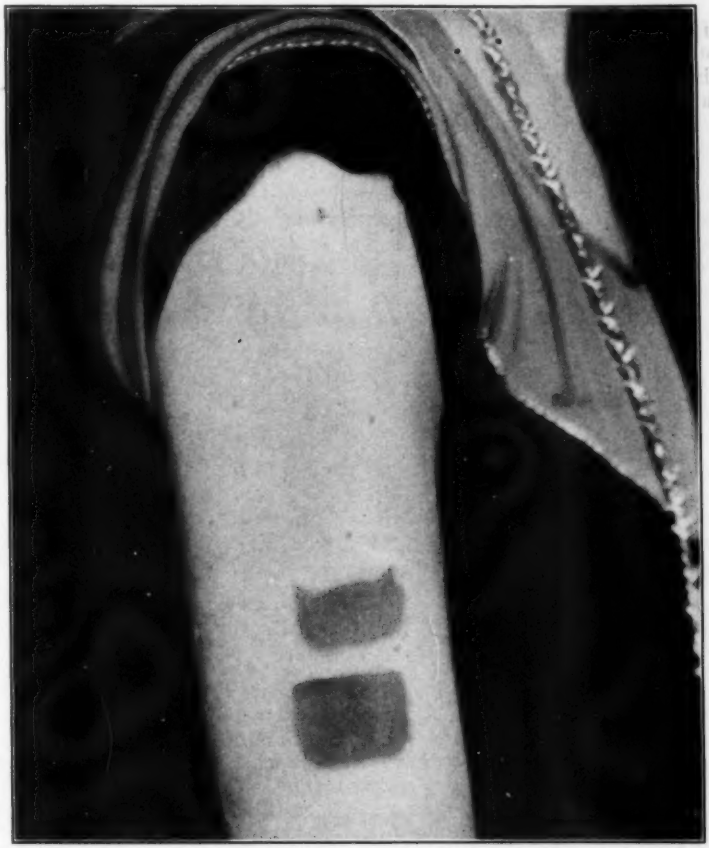

FIG. 2.—Two burns obtained with lamp; control (above), and through wool fabric (below). Ratio 20 to 1

burn on the skin both in direct sunlight and under the quartz mercury arc lamp.

The spectrographs of these four fabrics showed that none of the fabrics absorbed any of the rays of the spectrum, the range of which was between the limits of 500 and 250 microns. The fabric caused a scattering of the rays through defraction, which was very decided in cases of long-time exposure. Tests of a printing-out paper made by means of the spectrograph proved that it is only slightly sensitive to

the shorter visible light rays and extremely sensitive to the ultra-violet rays, line 313 being the most distinct. This line is among the ones most effective in antirachitic qualities as proved by the work of Hausser and Vahle and shown in their chart on "Skin sensibility to ultra-violet rays." [3]

OTHER FABRICS TESTED

Many other cotton fabrics of different weights were tested, and in each case the ratio of protection secured by screening printing-out paper from the rays of the lamp proved the correct ratio for time of exposure to produce burns on the skin both in direct sunlight and under the lamp. This was also true of all linen fabrics tested. In all cases this ratio was smaller than the ratio of the interspace between the threads to the space covered by the threads.

Two of the wool fabrics included in the test required a ratio of 20 to 1 for time of exposure to produce a burn, as shown in Table 6, while the third piece of wool, which was very much lighter, required a ratio of only 10 to 1; this, it will be noticed, is slightly more than double the ratio obtained by ultra-violet light and the sensitized paper.

TABLE 6.—*Coefficient of protection of additional textile fabrics*

Fabric	Coefficient determined by—			
	Photometer	Measurement of interspace	Action of ultra-violet rays on sensitized paper	Action of sunlight on skin
True silk	3.606	13.60	4.00	5.00
Do	3.844	19.10	6.00	12.00
Wild silk	4.850	14.59	8.50	10.00
Do	4.270	10.27	7.50	9.00
Wool	4.863	11.90	8.50	20.00
Do	4.940	10.40	9.00	20.00
Do	3,920	11.64	5.00	10.00
Cotton	5,280		3.00	3.00
Linen	3,360	4.04	2.33	2.33

The silk fabrics, of which four more were listed, two of wild silk and two of cultivated silk, gave varied results as a group. The individual fabrics gave the same results on repeated exposure, but no definite group results were obtained with silk as with the other fibers. In no case was the ratio of time of exposure necessary to secure a burn of the skin through silk less than the ratio of protection obtained by means of the lamp and the printing-out paper used.

Athough they varied widely the ratios indicated roughly the time of exposure to be employed when subject was under test. The ratio of time of exposure obtained by means of lamp and sensitized paper proved correct for time of exposure for all linen and cotton fabrics tested, while in the case of silk and wool fabrics the ratio obtained by this method was in every case less than the ratio required to secure a good burn.

[3] HAUSSER, K. W., and VAHLE, W. DIE ABHÄNGIGKEIT DES LICHTERYTHEMS UND DER PIGMENTBILDUNG VON DER SCHWINGUNGSZAHL (WELLENLÄNGE) DER ERREGENDEN STRAHLUNG. Strahlentherape 13: [41]-71, illus. 1922.

Tests proved that the skin burned in a shorter time of exposure to ultra-violet rays when the blood had been brought to the surface by application of heat. Such comparisons were obtained by exposing controls while the arm was still cool and comparing them with controls exposed after the arm had become warm from heat rays or from the application of heat to the arm.

CONCLUSIONS

The data submitted from a number of tests tend to prove that the protection from sunburn afforded the skin by fabrics depends primarily upon the per cent of interspace due to weave but that the vegetable fibers, cotton and linen, transmit some of the rays that burn and tan, thus offering a small coefficient of protection, whereas the animal fibers, silk and wool, absorb a larger portion of these rays, thus offering a higher coefficient of protection.

Due to the fact that temperature is a factor in the burning of the skin the greater conductivity of cotton and linen, as compared with that of silk and wool, might in a measure account for the higher prospective value of silk and wool.

SOME NITROGENOUS CONSTITUENTS OF CORN POLLEN[1]

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HISTORICAL

The first work recorded on the chemical analysis of pollen was by Fourcroy and Vauquelin (*1*),[2] who studied the ash and organic constituents of pollen of the date palm, *Phoenix dactylifera*. They found that this pollen contained a substance analogous to gluten, and a large amount of malic acid. Link (*9, p. 145*) reported much tannin and gluten in the pollen of the hazel tree. Vauquelin (*9, p. 237*) found sugar, resin, and tannin in stamens of the horse chestnut. Braconnot (*6*) reported the presence of potassium malate and a little nitrogenous material in the pollen of *Typha latifolia*.

Planta (*35*) found sucrose, starch, and 4.81 per cent nitrogen in the pollen of *Corylus avellana*. An extract of this pollen with 10 per cent NaCl solution gave a coagulum on heating, and peptones were also present. In the pollen of *Pinus sylvestris*, Planta (*36*) found 2.65 per cent nitrogen. Peptones, guanine, and hypoxanthine were present. An extract of the pollen with 10 per cent NaCl solution did not, however, give a coagulum on heating. Schulze and Planta (*39*) found a nitrogenous substance in the pollen of Corylus and pine. This substance yielded guanine and glucose, hence was called guanosin. Przybytek and Famintzin (*37*) found 2.4 per cent nitrogen in the pollen of *Pinus sylvestris*, and Kresling (*25*) reported tartaric and malic acid in the same kind of pollen.

Stift (*41*), working on pollen of the sugar beet, found the total nitrogen to be 3.6 per cent, protein nitrogen 2.66 per cent, and lecithin 1.57 per cent. Stoklasa (*42*) reported 5.16 per cent lecithin in pollen of the horse chestnut, and 5.86 per cent in that of the apple. Kammann (*21*) found that nitrogen containing organic substances made up 58 per cent of the total organic material of rye pollen, and of this 40 per cent was protein in nature and 18 per cent nonprotein. Winterstein and Hiestand (*50*) found in the pollen of *Alnus viridis* and *Pinus montana* a phosphatide which contained lecithin in combination with a complex carbohydrate.

Koessler (*24*) found the following nitrogen distribution in ragweed pollen:

	Per cent
Total nitrogen	4. 73
Nitrogen soluble in 5 per cent NaCl solution	1. 89
Nitrogen soluble in 5 per cent NaCl solution and coagulable	. 10
Nitrogen soluble in 5 per cent NaCl solution and noncoagulable	1. 66
Nitrogen soluble in 75 per cent alcohol after 5 per cent NaCl extraction	1. 57
Nitrogen in the insoluble residue	1. 35

[1] Received for publication Mar. 14, 1927; issued September, 1927. Published with the approval of the director as Paper No. 675, Journal Series, Minnesota Agricultural Experiment Station. Condensed from a thesis presented by C. G. Vinson to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy, Ross Aiken Gortner, major adviser.

[2] Reference is made by number (italic) to "Literature cited," p. 275.

Heyl has done the most extensive work on the chemical analysis of pollen. He found (*13*) that the exine was removed largely by extracting with ether and then alcohol; but that this did not remove the intine which is largely cellulose. Extracting with ether in a Soxhlet apparatus removed 13.26 per cent of the solids, and alcohol following the ether extraction removed an additional 20.9 per cent; but hot 95 per cent alcohol alone extracted 42.9 per cent of the solids. The pollen contained 4.99 per cent nitrogen, of which 1.08 per cent was alcohol soluble, 2.6 per cent was soluble in 10 per cent NaCl solution, and 0.8 to 1.1 per cent was extracted by 0.2 per cent alkali after extracting with 10 per cent NaCl. The residue from the various extractions contained 1.2 per cent nitrogen. In other papers Heyl (*16*, *17*) reports the nonnitrogenous constituents extracted by alcohol and ether. Ragweed pollen (*14*), which had been exhausted with ether and 95 per cent alcohol and then extracted with distilled water, gave 1.22 per cent albumin, coagulating at 65° C., and 1.1 per cent proteose. After extracting with distilled water and then with 10 per cent NaCl, dilute alkali extracted a protein which amounted to 2.9 per cent of the original material. This protein was precipitated on acidification, and was the chief protein present. The percentage of certain amino acids in these proteins has been reported by Heyl and Hopkins (*18*). Heyl (*15*) found that the coloring substances of ragweed pollen belong to the flavonols and are entirely glucosidic. Quercetin glucoside having the composition of $C_{21}H_{20}O_{12}$ was found. A glucoside of isorhamnetin was also obtained.

Corn pollen, however, has not received so much attention as ragweed pollen. Miyake (*28*) found phytosterol and inosite in corn pollen. Miyake (*29*) later identified adenine and choline. Anderson and Kulp (*3*), working on corn pollen, found 4.3 per cent nitrogen in the air-dry pollen. Phytosterol palmitate was identified. Inosite was found to be present to the extent of 0.83 per cent, choline 0.34 per cent, and l-proline to the extent of 0.6 per cent.

Anderson (*2*) found a saturated hydrocarbon, probably nonakosane, attached to the phytosterol palmitate from corn pollen. The phytosterol preparations differed, however, from ordinary phytosterol.

MATERIAL

Since no particular attempt had been made to identify the nitrogenous constituents of corn pollen, it was decided to concentrate on the nitrogenous fraction. About 4 kgm. of air-dry pollen, collected in 1917 and 1918, were available. The pollen was quite clean, as it had been screened through bolting-cloth, thus freeing it from anther sacs, filaments, etc. The air-dry pollen had a nitrogen content of 3.6 per cent and a moisture content of 3.97 per cent, as determined by bringing a sample to constant weight at room temperature in vacuo over H_2SO_4, as did Anderson and Kulp (*3*). Corn belongs to the anemophilous class of pollinated plants, and agrees with the findings of Lidforss (*26*) in that the nitrogen content of its pollen is relatively low as compared with pollen of entomophilous flowers.

According to Heyl (*14*), the walls (exine and intine) of the pollen grain constitute 65 per cent of the structure. The exine is relatively thick and resistant to mechanical disintegration. Planta (*35*) found that grinding pollen between grooved steel plates had no effect on

the grains. Winterstein and Hiestand (*50*) state that it is difficult to extract the entire grains, so part was crushed in a mortar and part ground in a mill. Anderson and Kulp (*3*) found that long grinding in a mortar, even after the pollen had been extracted with ether and alcohol, produces only a small percentage of broken cells. Realizing that a better extraction could probably be obtained with the pollen grains pulverized, the writer placed 313 gm. of the air-dry pollen in a ball mill of 4,000 c. c. capacity, and at the end of 24 hours grinding not an entire pollen grain could be found when a sample was examined under the microscope. After the ball mill had proved so efficient in pulverizing the grains, practically all the pollen was put through it.

PRELIMINARY EXPERIMENTS

Ten grams each of the milled and unmilled pollen were extracted with two 75 c. c. portions of ether in a Soxhlet apparatus, allowing 12 hours to each extraction. This was followed by extraction with two 150 c. c. portions of absolute alcohol in a 300 c. c. Florence flask, allowing 12 hours to each extraction. From the milled sample 3.0200 gm. of dry matter containing 0.0300 gm. of nitrogen were extracted, and from the unmilled 3.0250 gm. containing 0.0330 gm. of nitrogen, indicating that the unground pollen was as readily extracted by ether and alcohol as was the ground material.

Again 10 gm. each of fresh milled and unmilled pollen were extracted 14 hours in the cold with 150 c. c. of 5 per cent K_2SO_4 solution. The filtered extracts were made up to volume and total nitrogen determined on aliquots. The extract from the unmilled pollen contained 0.1455 gm. of nitrogen and that from the milled sample contained 0.1765 gm. of nitrogen, showing that the nitrogen was more readily extracted from the milled than the unmilled pollen. Approximately 50 per cent of the nitrogen was extracted from the milled pollen by the K_2SO_4 solution.

The residue of the unmilled pollen from the above extraction with 5 per cent K_2SO_4 solution was extracted 15 hours at room temperature with 150 c. c. of 5 per cent H_2SO_4. After filtering and making the filtrate up to volume, it was found to contain 0.0525 gm. of nitrogen. The residue from this extraction was then treated with 150 c. c. of 0.2 per cent NaOH, but after mixing, the reaction was neutral to litmus. Accordingly, sufficient additional dilute NaOH solution was added to give an alkaline reaction to litmus. After extracting for 15 hours the material was filtered by suction, washed, and the filtrate made up to volume. The total nitrogen in this extract was found to be 0.0512 gm. Thus the total nitrogen successively extracted from a sample of unmilled pollen by 5 per cent K_2SO_4, 5 per cent H_2SO_4, and then dilute alkali was $0.1455+0.0525+0.0512=0.2492$ gm. This is 2.49 per cent of the original air-dry pollen; hence the total nitrogen not extractable with aqueous solutions $=3.60-2.49=1.11$ per cent. Heyl (*13*) found 1.2 per cent nitrogen in the residue of ragweed pollen from similar extractions.

The residue from each of the above samples which had been extracted with ether and boiling absolute alcohol was extracted in the cold with 150 c. c. of 5 per cent K_2SO_4 solution for 14 hours. On filtering and making the filtrates up to volume, that from the milled

sample was found to contain only 0.036 gm. of nitrogen and that from the unmilled sample 0.046 gm. Apparently the ether and boiling alcohol made the remaining nitrogen less soluble, and the more intimate contact of the ether and alcohol with the ground pollen rendered a greater amount of the remaining nitrogen insoluble than in the case of the unground pollen. This precluded the possibility of extracting the pollen with ether and boiling alcohol before extracting the nitrogenous fractions with aqueous solutions. Heyl (*14*), however, found that in the case of ragweed pollen exhaustion with ether and alcohol at ordinary temperatures did not render the remaining nitrogen less soluble.

Ten gm. of milled pollen were extracted 24 hours with 150 c. c. of distilled water, the pollen residue centrifuged off, and the supernatant liquid then filtered. The pollen residue was then treated with 75 c. c. of distilled water, centrifuged, and the washings filtered. The filtrates were combined, made up to volume, and found to contain 0.129 gm. of nitrogen. The 5 per cent K_2SO_4 extraction therefore removed $0.1765-0.1290=0.0475$ gm. more of nitrogen than the distilled water alone. Therefore, to determine if a globulin was present, one 10-gram sample of milled pollen was extracted 14 hours with 100 c. c. of distilled water and another 10-gram sample of the milled pollen was extracted for a like period with 5 per cent K_2SO_4. The extracts were centrifuged and then filtered. Each was gradually heated. An opalescence began to form at 75° C. and a slight coagulum appeared at 80°. On heating each to boiling under a reflux for an hour, then filtering, 0.0346 gm. of coagulum was obtained from the aqueous extract and 0.0556 from the 5 per cent K_2SO_4 extract. Five gm. of K_2SO_4 were then added to the aqueous extract and the solution again heated to boiling under a reflux for one hour. Another coagulum weighing 0.0294 gm. was obtained, making a total of 0.063 gm. of coagulum from the distilled water extract, which coagulum contained 0.0056 gm. of nitrogen, while that from the 5 per cent K_2SO_4 extract contained 0.0053 gm. of nitrogen. The distilled water had, therefore, removed as much of the heat-coagulable nitrogenous material as the 5 per cent K_2SO_4, and, therefore, the presence of a globulin was improbable, since the salt content of the pollen would hardly give a high enough concentration to hold globulins in solution. Heyl (*14*) found, however, that salt extraction removed more of the heat coagulable fraction from ragweed pollen than extraction with distilled water.

ENZYMES

On warm days when the hand came in contact with the pollen the small amount of perspiration on the fingers seemed to cause the pollen to partially autolyze and form a waxy paste. If this was a case of autolysis, then enzymes must have been present. Kammann (*22*) found proteose in rye pollen. Paton (*32*) working on the pollen of Easter lily, red maple, Norway maple, Siberian crab apple, Austrian pine, magnolia, dandelion, goldenrod, ragweed, and corn, found erepsin, pepsin, and trypsin in some. Trypsin and pepsin (*33*) were found in corn pollen. Hence it was quite necessary to determine whether proteolytic enzymes were present before attempting to isolate the nitrogenous fractions. In order to try to determine the presence of active enzymes, ten 5-gram samples of

the milled pollen were placed in 10 separate 250 c. c. Erlenmeyer flasks, and the flasks were then plugged with cotton and allowed to stand in an incubator at 35° C. overnight. The next morning 100 c. c. of distilled water, which had attained the same temperature by standing in the incubator overnight, were added to each of the 10 flasks, rotating the flasks to secure thorough wetting of the pollen, and 2 c. c. of toluene were added. The flasks were then agitated at half-hour intervals, except at night. The water was added at 7.30 a. m. The first flask was removed at 8 a. m.; the second at 10 a. m.; the third at 12.30 p. m.; the fourth at 3 p. m.; the fifth at 7 p. m.; the sixth at 8 a. m. the second day; the seventh at 4 p. m. the second day; the eighth at 8 a. m. the third day; the ninth at 8 a. m. the fourth day; and the tenth at 8 a. m. the fifth day. On being removed from the incubator each sample was made up to 150 c. c., 50 c. c. of which were used for the $SnCl_2$ precipitation [3] and another 50 c. c. for the copper precipitation.[3] After precipitating the proteins in the two latter fractions with $SnCl_2$ and copper reagents, respectively, each was made up to 100 c. c., centrifuged, filtered, and total nitrogen determined on 25 c. c. samples and amino nitrogen determined on 2 c. c. samples. The 50 c. c. remaining, after taking the samples for $SnCl_2$ and copper precipitation, were filtered, and total nitrogen was determined on 25 c. c. samples and reducing sugars on 5 c. c. samples. Table 1 shows the data which were obtained.

TABLE 1.—*Autolysis of corn pollen (5-gram samples)*

Sample No.	Total nitrogen in the entire aqueous extract	Total nitrogen not precipitated by $SnCl_2$	Total amino nitrogen not precipitated by $SnCl_2$	Total nitrogen not precipitated by copper	Total amino nitrogen not precipitated by copper	Total reducing sugars
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Milligrams*
1	0.055	0.025	0.002	0.020	0.008	48.76
2	.056	.032	.006	.025	.006	65.76
3	.056	.036	.003	.036	.002	72.75
4	.059	.027	.000	.031	.000	77.51
5	.066	.027	.002	.039	.002	77.83
6	.064	.034	.005	.039	.001	80.05
7	.063	.025	.005	.043	.006	82.50
8	.062	.037	.006	.044	.004	85.77
9	.071	.033	.016	.039	.010	88.31
10	.075	.036	.016	.043	.016	91.17

The precipitation with copper as recommended by Olsen [4] was much less satisfactory than with the $SnCl_2$ reagent, owing to the fact that the end point with phenolphthalein was obscured by the color of the solution. The same difficulty was also experienced in using bromcresol purple as an indicator in the $SnCl_2$ precipitation.

The small amount of amino nitrogen present renders these determinations subject to great error, and perhaps of little value. It is evident, however, that the total nitrogen in the filtrates from the two precipitations did not increase to any appreciable extent after

[3] OLSEN, A. G. A STUDY OF THE PROTEASES OF BREAD YEAST, SACCHAROMYCES CEREVISIAE. [Unpublished master's thesis. Copy on file, Div. Agr. Biochem., Univ. Minn., St. Paul.] Part of the data in this thesis has been published (*51*).

[4] Olsen, A. G. Op. cit.

the first two hours. The total reducing sugars, however, show a steady increase, as might be expected from the reports of Van Tieghem (*45*), Erlenmeyer (*10*), and Green (*12*), working on the pollens of other species; hence carbohydrases were present and active.

From the above data it was evident that extractions of the pollen could be made with aqueous solutions without danger of alteration of the nitrogenous fractions by proteolytic enzymes.

PRELIMINARY SURVEY

For preliminary survey work, 500 gm. of the milled pollen were extracted 20 hours with 3,000 c. c. of distilled water. Fifteen hundred cubic centimeters of the water were first added, but after agitation and standing 15 minutes the entire mass set to quite a stiff gel. Davison (unpublished data) later found a very active pectase in this sample of pollen, which may explain the gel formation. Lloyd (*27*) has shown that the pollen tubes may show great variation in the amount of water which they will absorb. This may be associated with a gel formation.

The aqueous extraction was carried out by keeping the material, which was in a 5-liter bottle, on a shaking machine for eight hours. The remainder of the time the bottle was shaken at intervals. At the end of 20 hours the material was passed through a supercentrifuge. The filtrate was then filtered through paper pulp in order to clarify it. The solution was then carefully concentrated in vacuo, keeping the temperature below 50° C. This solution was acid to Congo red in reaction, and on attempting to coagulate the heat-coagulable proteins by boiling, only a slight turbidity developed. On testing for albumin by the method of Sörensen (*40, p. 28*) the turbidity increased only slightly. This indicated the presence of very little albumin in the corn pollen. Heyl (*14*), however, found 1.22 per cent of albumin in the aqueous extract of ragweed pollen.

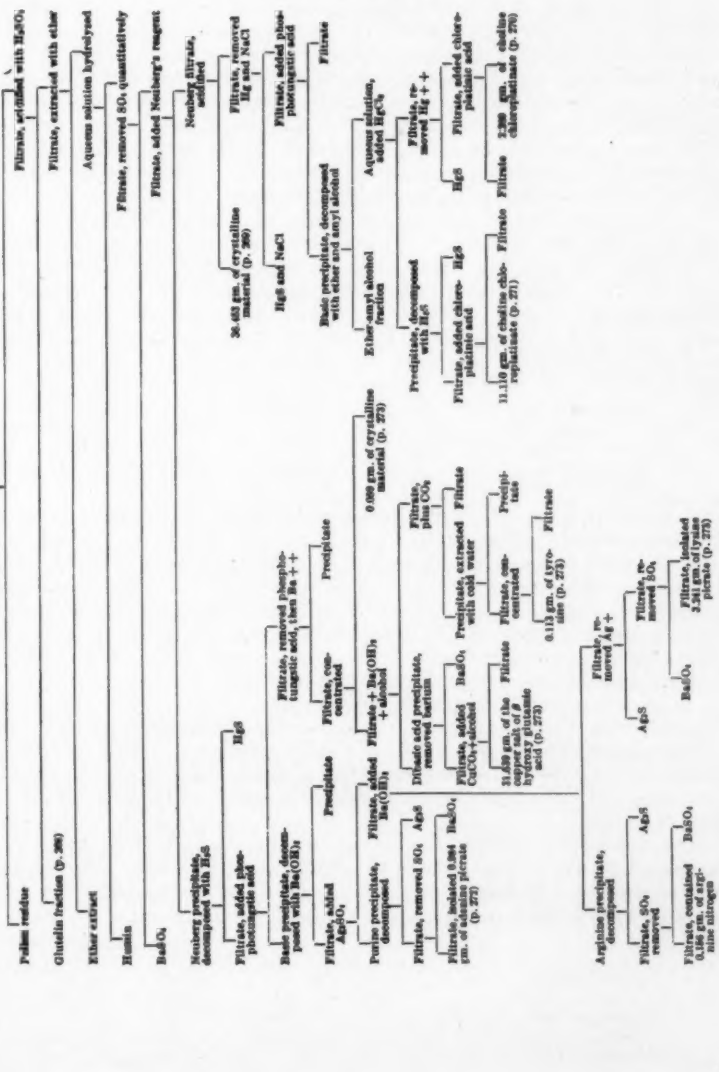
The pollen residue from the above aqueous extraction was extracted with 2,000 c. c. of 0.2 per cent NaOH solution for 24 hours, being kept on a shaking machine for eight hours. This material was likewise put through the supercentrifuge, as it was impossible to filter by gravity or suction. The ordinary centrifuge could not be used, as much of the fine material could not be thrown down with this type of centrifuge. The filtrate was dark in color and quite viscous. It was filtered, and a gelatinous precipitate filtered off. The reaction of the filtrate to litmus was neutral; so evidently not enough alkali had been used to keep the material in solution. The gelatinous precipitate was extracted with 3,000 c. c. of a 0.4 per cent NaOH solution, and on filtering only a negligible residue remained. The filtrate was very dark in color and on being made acid to litmus, but alkaline to Congo red, by carefully adding 5 $N.H_2SO_4$ a gray-colored flocculent precipitate settled out, leaving a clear amber-colored supernatant liquid.

The gray precipitate was extracted 24 hours at room temperature with 1,000 c. c. of 5 per cent K_2SO_4 solution, remaining on the shaking machine eight hours. The solution was filtered, and the filtrate plus the washings concentrated and made up to 1,000 c. c. It was found to contain about 0.100 gm. of nitrogen. If this latter extract represented globulin, certainly there was very little present in the pollen worked with. Heyl (*14*) found an albumin in ragweed pollen,

but does not report the presence of globulins. Bauman, Chudnoff, and Mackenzie (*5*), however, speak of the globulin fraction. The precipitate obtained on neutralizing the alkaline extract is termed the glutelin fraction.

MAIN EXPERIMENT

OUTLINE OF PROCEDURE



One kilogram of the ground pollen was placed in a 5-liter bottle and 3,000 c. c. of distilled water added. In using 0.2 per cent NaOH solution on the preliminary sample, the resulting solution was neutral to litmus; consequently, in this case, 15 per cent NaOH solution was added until the mixture gave an alkaline reaction to litmus, 200 c. c. of the alkali solution being required. The pollen was extracted 24 hours, being kept on a shaking machine for 10 hours. A like extraction was made of another kilogram sample, and this plus the 500 gm. used in the preliminary work made 2½ kgm. extracted. At the end of each extraction the material was passed through a supercentrifuge, and then the filtrates were combined. The filtrate from the above extractions was alkaline to litmus, but not to phenolphthalein. The pollen residue when suspended in 3,000 c. c. of distilled water again gave a neutral reaction to litmus. This suspension was made alkaline to litmus with NaOH solution, and allowed to stand 14 hours with shaking at intervals. At the end of the extraction the material was centrifuged as before.

Upon adding the second extract to the first the combined extracts gave an alkaline reaction to phenolphthalein. By carefully adding $5N.H_2SO_4$ the reaction was carried to that point where all material was yet in solution but precipitation would take place with only a slight amount of acid. The solution was then concentrated in vacuo at a very low temperature. The solution (No. 11) was made up to 8,000 c. c. and the following data obtained from its analysis:

	Grams
Total nitrogen	51. 412
Ammonia nitrogen	. 440
Amide nitrogen	1. 840
Amino nitrogen	8. 444
Organic solids	816. 64

After aliquoting, $5N.H_2SO_4$ was slowly added to the remainder of the concentrated extract until it was just acid to Congo red, when a gray-colored precipitate formed. This precipitate was light, and tended to come to the top of the liquid. By adding ether and rapidly stirring the liquid it was found that the precipitate would form a uniform layer at the top from which it could be siphoned off into ordinary centrifuge tubes and a further separation obtained by centrifuging in the ordinary type of centrifuge.

After separating the glutelin precipitate in this fashion it was extracted with 2 liters of ether, to remove fat-soluble materials. The precipitate was then dissolved in 6 liters of slightly alkaline water, and reprecipitated by acidifying with acetic acid. The dry precipitate weighed 248.1 gm., contained 22.461 gm. of nitrogen and 19.97 gm. of ash.

The combined filtrates from the glutelin precipitation were concentrated in vacuo and extracted with 2 liters of ether. The aqueous solution was then concentrated, made up to volume (solution 12), aliquoted, and the following data obtained on analysis:

	Grams	Per cent of solution 11
Total nitrogen	25. 790	52. 68
Ammonia nitrogen	. 320	72. 82
Amide nitrogen	. 721	39. 18
Amino nitrogen	7. 372	83. 71
Organic solids	716. 400	87. 73

As one would expect, the greater amount of the amino nitrogen came down in the filtrate from the glutelin, and by difference the greater amount of the amide nitrogen remained in the glutelin fraction.

The remainder of solution 12 was hydrolyzed 24 hours with 20 per cent H_2SO_4. The humin from this hydrolysis weighed 49.22 gm. and contained 1.942 gm. of nitrogen. Sulphate was exactly removed with $Ba(OH)_2$. After precipitation was complete the solution containing the $BaSO_4$ was heated and kept almost at the boiling point for about two hours, then allowed to cool slowly. In this way the $BaSO_4$ precipitated in relatively coarse particles, and the solution then filtered fairly readily and a relatively small amount of nitrogen was lost by adsorption. The $BaSO_4$ weighed, when dry, 3,712 gm. and contained 0.989 gm. of nitrogen, which is a low figure for nitrogen in such a large amount of precipitate.

The filtrate and washings from the $BaSO_4$ precipitate were concentrated, made up to volume (solution 13), aliquoted, and on analysis, gave the following:

	Grams
Total nitrogen	25.270
Ammonia nitrogen	1.477
Amino nitrogen	10.351
Organic solids	602.190

PRECIPITATION WITH NEUBERG AND KERB'S REAGENT

The remainder of solution 13 was concentrated, placed in a large precipitating jar, and precipitation with Neuberg and Kerb's (*30*) reagent carried out. Solutions containing 600 gm. of mercuric acetate and 215 gm. of Na_2CO_3 were added until the volume reached 6 liters and no end point was indicated. It was then considered advisable to add an equal volume of alcohol, remove the precipitate, acidify and concentrate the filtrate, and repeat the Neuberg precipitation at greater concentration, as this reagent is supposed to act best when the amino acid concentration is 10 to 20 per cent. In the second precipitation 195 gm. of mercuric acetate and 60 gm. of Na_2CO_3 were added, and still no orange-red precipitate was obtained on adding either of the two reagents. The failure to observe the characteristic colored precipitate at the end point may have been due to the color of the solution obscuring it, as in the first precipitation an orange-red precipitate collected on the stirring rod, and the last precipitate had a yellow-red layer on top. The last precipitate was very small. Both precipitations were carried out in the cold, the precipitating jar being surrounded by running water which was at a temperature of 10° C. In each case after adding the Neuberg reagent an equal volume of alcohol was added and the mixture then allowed to stand overnight. The two precipitates were combined and washed with 1,000 c. c. of 80 per cent alcohol. On acidifying the filtrate from the first precipitate with acetic acid, a flocculent precipitate formed which was filtered off and was found to be crystalline. It weighed 36.453 gm. and had a distinctly bright, metallic luster. It contained neither nitrogen nor sulphur. This substance is reserved for further investigation.

The filtrate from the last Neuberg precipitate was acidified, concentrated, and mercury was then removed as HgS. The filtrate and washings from the HgS were concentrated and NaCl removed

with concentrated HCl and alcohol. The filtrate from the NaCl was concentrated and made up to volume (solution 15), aliquoted, and the following figures obtained on analysis:

	Grams
Total nitrogen	11. 127
Ammonia nitrogen	. 669
Amino nitrogen	2. 164
Organic solids	266. 325

PRECIPITATION OF BASES FROM THE NEUBERG FILTRATE

Bases were precipitated from the remainder of solution 15 by adding 5 per cent by weight of HCl and then adding a 20 per cent solution of phosphotungstic acid in 5 per cent HCl until precipitation was complete. After standing in the cold overnight, the precipitate was filtered off, and washed with a 2.5 per cent solution of phosphotungstic acid in 5 per cent HCl. Phosphotungstic acid was then removed from the basic precipitate with amyl alcohol and ether according to the method of Van Slyke (*44*) and Jacobs (*20*). The precipitate was suspended in water in a large Erlenmeyer flask, acidified with concentrated HCl, a mixture of equal parts of amyl alcohol and ether was added, and the flask agitated. It was necessary to filter twice before obtaining a good boundary between the two liquids the first time. Three extractions with the amyl alcohol–ether mixture were sufficient to remove all phosphotungstic acid from the aqueous solution so that no precipitate was obtained on adding a drop of a saturated solution of $Ba(OH)_2$. A small amount of the precipitate failed to go into solution. This was ground up with baryta and water and yet failed to go into solution. The alkaline extract thus obtained was very dark in color, and on being acidified gave a precipitate of colored material. It contained a total of 0.082 gm. of nitrogen. It is believed the insoluble part was largely humin.

BASES OF THE NEUBERG FILTRATE

The basic fraction as precipitated from the Neuberg filtrate (solution 24) with phosphotungstic acid contained 3.587 gm. of nitrogen, 0.2989 gm. of which was animo nitrogen, showing that only about 14 per cent of the amino nitrogen of the Neuberg filtrate was basic nitrogen. Schulze's (*38*) method was accordingly used to separate choline from other plant bases. Mercuric chloride in boiling aqueous solution was added to the remaining portion of solution 24 until no further precipitate was obtained (*4*), and the mixture was allowed to stand overnight in the cold before filtering. The precipitate was extracted with boiling water, the insoluble portion was filtered off, and the two filtrates were combined. Mercury was removed as HgS, and alcohol was added to the concentrated filtrate from the HgS. A precipitate weighing 8.849 gm. and containing 0.2159 gm. of nitrogen formed at this point. This consisted of a mixture of ammonium and sodium chloride. The filtrate from the above precipitate was made up to volume and on analysis was found to contain 1.825 gm. of nitrogen. To an aliquot of this solution a 5 per cent platinic chloride solution was added until precipitation was complete. The chloroplatinate obtained weighed 0.4538 gm. and contained 31.84 per cent platinum; hence it must have been choline chloroplatinate.

The insoluble mercury compound was suspended in water and decomposed with H_2S. The filtrate from the HgS was taken down to dryness on a water bath. The residue was dissolved in a little hot absolute alcohol and again concentrated to dryness in vacuo. Very large needle-shaped crystals formed. The crystals were dissolved in absolute alcohol with warming and made up to 250 c. c. (solution 33). This solution contained 1.005 gms. of nitrogen. To 50 c. c. of this solution platinic chloride was added until precipitation was complete. A total of 2.222 gm. of chloroplatinate were obtained from this aliquot, thus giving a total chloroplatinate obtainable from the solution of 11.110 gm. This chloroplatinate was found to contain 31.73 per cent of platinum; hence it must have been choline chloroplatinate as the theoretical platinum content for choline chloroplatinate is 31.68 per cent. From the betaine fraction, as precipitated from the Neuberg filtrate, a total of 13.379 gms. of choline chloroplatinate were thus obtainable, representing 0.608 gm. of nitrogen.

THE NEUBERG PRECIPITATE

The Neuberg precipitate was brought into suspension in water, acidified with acetic acid, and decomposed with H_2S. The filtrate and washings from the HgS were concentrated and made up to volume (solution 14), aliquoted, and on analysis found to contain:

	Grams
Total nitrogen	11. 810
Ammonia nitrogen	2. 058
Amino nitrogen	3. 980
Organic solids	95. 720

Following the use of Neuberg's reagent the ammonia nitrogen increased 84.63 per cent over that in solution 13, and the amino nitrogen decreased 40.64 per cent. In some similar work reported by Vickery and Vinson (*48*), following the use of Neuberg's reagent, the ammonia nitrogen increased 7.3 per cent and the amino nitrogen decreased 15.53 per cent. It is entirely possible that the poor results in precipitating amino nitrogen with Neuberg's reagent were due to adding an excess of the reagent; for mercury is known to be a very effective catalyst, and an excess of the mercuric acetate may bring about a decrease in the amino nitrogen by catalyzing deamination and ring formation.

BASIC FRACTION OF THE NEUBERG PRECIPITATE

Bases were precipitated by phosphotungstic acid from the fraction precipitated by Neuberg's reagent. The filtrate from the basic fraction, after the removal of phosphotungstic acid with $Ba(OH)_2$ and subsequent removal of the barium with H_2SO_4, was found to contain 4.292 gm. of nitrogen, 2.811 gm. of which were amino nitrogen. On finding the ratio of amino to total nitrogen so low, the solution was again hydrolyzed for 10 hours with 10 per cent by weight of H_2SO_4. After removing the H_2SO_4 quantitatively the solution was found to contain 4.292 gm. of nitrogen, 3.505 gm. of which were amino nitrogen; thus, due to hydrolysis, the amino nitrogen was increased 24.7 per cent. Bases were again precipitated, and the precipitate obtained added to the main phosphotungstate precipitate, which was then decomposed with $Ba(OH)_2$. The filtrate from the barium phos-

photungstate precipitate, after removal of barium was found to contain:

	Grams
Total nitrogen	3.274
Ammonia nitrogen	.291
Amino nitrogen	1.133
Organic solids	20.610

The ammonia nitrogen in the above was probably a volatile amine, in part at least, for in titrating the distillate after ammonia determinations an amine odor could be detected.

Separation of the bases was carried out as described by Vickery (*46*) adding Ag_2SO_4 in boiling saturated aqueous solution until a brownish yellow precipitate was obtained, when a drop of the solution was added to a drop of saturated $Ba(OH)_2$ solution. The purine precipitate was decomposed with H_2S, and the filtrate from the Ag_2S, after removal of SO_4, was found to contain 0.317 gm. of nitrogen. Picric acid, in saturated aqueous solution, was then added until precipitation was complete. The dry weight of the picrate thus obtained was 0.984 gm., and its melting point was 278° C. (uncorrected). Picric acid was removed from the above picrate and the purine converted into the sulphate, as described by Vickery and Leavenworth (*47*). Crystals of the sulphate formed, but these were small. Picric acid was then added to the solution of the sulphate, and a picrate melting point 281° C. was obtained. This is the melting point of adenine picrate, and thus 0.199 gm. of nitrogen as adenine was accounted for.

ARGENINE FRACTION

The filtrate and washings from the purine precipitate were saturated with finely divided baryta. A buff-colored precipitate formed. The precipitate was decomposed with H_2S, and H_2SO_4 in the filtrate from the Ag_2S was exactly removed. The filtrate from the $BaSO_4$ was found to contain 0.546 gm. of nitrogen. Arginine nitrogen in this fraction was determined by the method of Van Slyke (*43*), using the apparatus devised by Holm (*19*). This gave an arginine nitrogen content of 0.156 gm.

LYSINE FRACTION

Silver was removed as Ag_2S from the filtrate and washings from the arginine fractions. Sulphate was then removed quantitatively with $Ba(OH)_2$. The filtrate from the $BaSO_4$ was found to contain 2.245 gm. of nitrogen. Lysine was precipitated from this solution by the method of Winterstein (*49*). The precipitate of the mercury salt thus obtained was centrifuged off and the filtrate found to contain only 0.202 gm. of nitrogen. The mercury salt was decomposed with H_2S, after being suspended in dilute H_2SO_4. The sulphate ion was then removed quantitatively, and the filtrate from the $BaSO_4$ concentrated to small volume, when small, yellow, rectangular crystals formed in abundance. Chlorides were then removed quantitatively with Ag_2SO_4, the excess silver removed as Ag_2S, and H_2SO_4 in the filtrate from the Ag_2S exactly removed. The filtrate and washings from the $BaSO_4$ were found to contain 1.797 gm. of nitrogen. This was concentrated to small volume, brought to the sensitive point with absolute alcohol, and a saturated solution of picric acid in ab-

solute alcohol was added until precipitation was complete. A total of 3.241 gm. of lysine picrate decomposing characteristically at 250° to 253° C. were obtained. This accounts for 0.254 gm. of nitrogen.

NONBASIC FRACTION OF THE NEUBERG PRECIPITATE

Phosphotungstic acid was removed from the filtrate of the bases from the Neuberg precipitate. The filtrate and washings from the barium phosphotungstate were concentrated and SO_4 removed quantitatively. The filtrate (solution 19) from the $BaSO_4$ on analysis was found to contain the following:

	Grams
Total nitrogen	3. 209
Ammonia nitrogen	0. 042
Amino nitrogen	2. 491
Organic solids	27. 379

The remainder of solution 19 was concentrated to small volume, when 0.069 gm. of crystalline material separated. Under the microscope the crystals were found to be large needles which were not collected in sheaves, as in the case of tyrosine, and, furthermore, the crystals were lemon yellow in color, and, dissolved in alkaline solution, gave a light green solution. The melting point was 348.8° C. (corrected). The melting point approximates that of apigenin or myricetin (*34*) but the other physical properties do not correspond. Some of the material was fused with sodium, and the Prussian-blue test found to be negative as well as the test for reduced sulphur with sodium nitroprusside. The crystals were only slightly soluble in acid, but were soluble in alcohol. It is believed that this material is a flavone or flavonol.

DIBASIC ACID FRACTION

The filtrate from the 0.069 gm. of crystalline material was concentrated to a sirup and dibasic acids removed by the method of Foreman (*11*), but varied according to the method of Kingston and Schryver (23), precipitating the dibasic acids as the barium instead of the calcium salts. The dibasic acid fraction so precipitated, after removal of barium contained 2.311 gm. of nitrogen. Beta hydroxy glutamic acid (*7*, *8*) was isolated from this fraction as the copper salt, precipitating it from aqueous solution with alcohol. On analyzing this salt the following data were obtained:

	Per cent	Percentage calculated for β OH glutamic acid
Copper content found	27. 78	28. 3
Total nitrogen found	6. 50	6. 23
Amino nitrogen found	6. 17	6. 23

A total of 31.639 gm. of the copper salt were actually isolated. This contained 1.972 gm. of nitrogen, corresponding to a weight of 22.96 gm. of the β hydroxy glutamic acid, which is 0.92 per cent of the original (2,500 gm.) pollen sample.

MONOAMINO—MONOCARBOXYLIC ACID FRACTION

Carbon dioxide was passed into the alcoholic filtrate from the precipitate of the barium salts of the dibasic acid fraction. This was carried out at 0°C., according to the directions of Kingston and Schryver (*23*). From the carbamino precipitate soluble in cold

water, 0.113 gm. of crystals in characteristic sheaves were obtained. The crystals gave Millon's reaction and contained 7.64 per cent nitrogen. These crystals were, therefore, tyrosine, as the theoretical nitrogen content of tyrosine is 7.74 per cent.

DISCUSSION

The heat-coagulable fraction of the aqueous extract from the pollen contained only about 10 per cent of nitrogen, whereas albumin is generally considered to contain about 16 per cent. Likewise, the glutelin fraction contained scarcely 10 per cent of nitrogen. Accordingly, these fractions are either highly contaminated with nonprotein material or else belong to the glycoproteins.

It is evident that corn pollen contains very little, if any, globulin. Extraction with salt solution failed to give any more heat-coagulable nitrogen-containing material than extraction with distilled water; and extraction of the glutelin fraction with salt solution gave only a negligible amount of nitrogen.

The use of Neuberg's reagent in precipitating amino nitrogen proved quite disappointing. This is the second experience in which this reagent has seemed to be instrumental in decreasing amino nitrogen, probably catalyzing ring formation. In clear solutions where the end point is easily observed, this reagent no doubt can be satisfactorily employed; but it is very evident that under some conditions it is not at all satisfactory.

Since Kingston and Schryver have shown that the barium salt of inactive aspartic acid is quite insoluble, it would seem more advisable to remove the dibasic acid fraction before precipitating the bases with phosphotungstic acid; for on making the filtrate from the bases alkaline to phenolphthalein with $Ba(OH)_2$ the inactive aspartic acid may be lost, thus accounting for some of the nitrogen in the barium phosphotungstate precipitate. Immediate removal of the dibasic acids after hydrolysis would seemingly be preferable, for with the strong acids and bases present, in concentrating to small volumes, some interaction is inevitable. This viewpoint is supported by the fact that on hydrolyzing the second time the amino nitrogen was increased 24.7 per cent, and another basic precipitate was obtained on adding phosphotungstic acid. With the early removal of the relatively strong dibasic acids the tendency for amino nitrogen to decrease would surely not be so great, especially when using Neuberg's reagent.

The 0.069 gm. of crystalline material (p. 273) is believed to be a flavone or flavonol pigment. This conclusion is drawn from the following facts: It is lemon yellow in color; nitrogen and sulphur are absent; the melting point is high; it changes color in alkaline solution; and is soluble in alcohol, but quite insoluble in acid or neutral aqueous solutions.

Beta hydroxy glutamic acid is the first dibasic amino acid ever reported as occurring in pollen of any kind. Planta states that asparagin could not be detected in the pollen of *Corylus avellana*. Schulze and Planta were unable to detect asparagin or glutamin in the pollen of Corylus or pine. Heyl, working with ragweed pollen, was unable to get copper salts to separate from the amino fraction after removal of bases. The dibasic acid fraction gave a very high

yield of β hydroxy glutamic acid as 85.33 per cent of the nitrogen of this fraction as accounted for in the copper salt actually isolated. Aspartic and glutamic acids were absent.

SUMMARY

A systematic study of the nitrogenous compounds contained in the alkali extract of corn pollen, and not precipitated on neutralization is reported.

The pollen was effectively pulverized by grinding in a porcelain ball mill.

The air-dry pollen contained 3.97 per cent of moisture and 3.6 per cent of nitrogen.

Successive extractions with distilled water, 5 per cent K_2SO_4, dilute H_2SO_4, then dilute alkali removed nitrogen to the extent of 2.49 per cent of the pollen, leaving 1.11 per cent nonextractable by the above solvents.

A substance behaving like a glutelin was isolated and was found to make up 9.9 per cent of the air-dry pollen. It contained 24.96 per cent of the total nitrogen in the air-dry pollen.

Proteolytic enzymes were not active, but active carbohydrases and a pectase were present.

Twenty-five hundred grams of air-dry pollen were extracted with dilute alkali and the filtrate neutralized with dilute H_2SO_4, when a precipitate formed. This precipitate is termed the glutelin fraction. The filtrate from the glutelin fraction, after extraction with ether, was hydrolyzed and the following substances determined:

a. 0.985 gm. of adenine picrate containing 0.199 gm. of nitrogen was isolated.

b. Arginine nitrogen was determined by the method of Van Slyke. This gave 0.156 gm. of nitrogen as arginine.

c. 3.241 gm. of lysine picrate were isolated.

d. 0.1129 gm. of tyrosine was obtained.

e. 31.639 gm. of the copper salt of β hydroxy glutamic acid were isolated. Aspartic and glutamic acids were not present.

f. 13.379 gm. of choline chloroplatinate were obtained, containing 0.608 gm. of choline nitrogen.

0.069 gm. of what is believed to be a flavone or flavonol was isolated.

On acidifying the Neuberg filtrate with acetic acid 36.453 gm. of a crystalline substance with metallic luster were obtained. This substance contained neither nitrogen nor sulphur, and is believed to be the mercury salt of the above flavonol. The identification of this compound is reserved for a later report.

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UTILIZATION OF THE GRAIN IN KAFIR AND CANE SILAGE BY DAIRY COWS[1]

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INTRODUCTION

Throughout the Southwest and along the southern border of the Corn Belt a large acreage of the sorghums is grown and used as silage in feeding dairy and beef cattle. During the course of a feeding experiment with dairy cows at the Oklahoma Experiment Station, it was observed that a considerable quantity of the grain in such silages is passed out into the manure apparently unused. Because of this loss many farmers practice heading these crops before ensiling them, grinding the heads separately, and feeding them in the concentrate portion of the ration. There is a question as to whether the loss of grain from the silage is sufficient to justify this practice being more widely recommended.

HISTORICAL

Cave and Fitch[2] found that as high as 90 per cent of the seeds in sorgo (sweet sorghum) silage pass through the cow undigested. The determinations were made by counting the number of seeds in aliquot samples of silage and of manure secured from two cows over a five-day period. As the result of this brief work these investigators raised the question as to whether it might not be advisable to remove the heads before ensiling this crop, and to use the ground heads in the grain ration.

In a feeding trial conducted by Aicher and McCampbell,[3] it was found that kafir and cane silages fed to steers in combination with two pounds of cottonseed meal daily had comparative feeding values as follows:

	With heads	Without heads
Kafir silage	72. 40	45. 31
Cane silage	69. 90	60. 60

Thompson[4 5] found after four years of feeding trials with swine that whole kafir was utilized less efficiently than ground kafir, and that these had relative feeding values on a numerical basis as follows:

	Whole kafir	Ground kafir
Self-fed	110	130
Hand-fed	100	111
Soaked, whole	87	
In the head (dry)	86	

[1] Received for publication Apr. 25, 1927; issued September, 1927.

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[5] Information to the authors. 1927.

He concluded that the feeding value of cane seed and of the grain sorghums was increased 10 to 25 per cent by grinding, since when fed in ground form a larger proportion of the grain was prevented from passing through the digestive tract apparently unused.

EXPERIMENTAL METHODS

Eight dairy cows were divided into two groups, one receiving kafir silage and the other cane silage. The cows were fed individually, each animal being allowed 30 pounds of silage and 10 pounds of alfalfa hay per 1,000 pounds live weight. The grain ration of corn meal, ground oats, wheat bran, and cottonseed meal was fed according to the milk and butterfat yields to meet the calculated requirements of the Morrison feeding standard. Refused feeds were reweighed and recorded.

A 20-day preliminary feeding period preceded the 10-day experimental period. During the latter period manure was collected from each cow daily. The cows remained in stanchions except when released for exercise, at which times an attendant was with them to collect all manure voided.

The cane and kafir grains voided by the individual cows were mechanically separated daily from the entire quantity of manure, by the use of screens and water. These recovered grains were dried, screened, fanned, and hand picked to remove all foreign material. Samples of these air-dry kernels were preserved for chemical analysis.

The grain from large quantities of cane and kafir silages was secured mechanically, by the use of sieves, water, and a fan to free the grain from all stalk and leaf material.

The dry weights of 1,000 hull-free kernels from the silage and from the manure were taken to determine whether any losses in weights of individual kernels had occurred during their passage through the cow's digestive tract.

Chemical analyses were made of the dry hull-free samples of grain obtained from the silage and from the manure, to determine by comparison what losses of nutrients had occurred in the kernels thus recovered from the manure.

PRESENTATION OF DATA

From 565 pounds of cane silage, 21 pounds of clean, air-dry grain were obtained. The air-dry grain constituted 3.72 per cent of the weight of fresh silage. Likewise, 611 pounds of fresh kafir silage contained 18.76 pounds of air-dry kafir grain, or 3.07 per cent of the weight of fresh kafir silage.

The first group of four cows consumed a net total of 1,374 pounds of cane silage, while the second group of four cows ate 1,364 pounds of kafir silage. One cow in the latter group was off feed. Exclusive of this animal, 1,096 pounds of kafir silage were consumed by the group. The first group consumed 51.11 pounds of grain in the form of whole cane seed in the silage, and of this amount 17.33 pounds, or 33.91 per cent, were recovered from the manure. Excluding the cow in the second group which was off feed, 33.65 pounds of kafir grain were consumed, of which 16.64 pounds, or 49.46 per cent, of kafir grain were found to have been voided in the manure. Data for the individual cows are presented in Table 1.

TABLE 1.—*Losses of grain into the manure, from cane and kafir silage consumed by dairy cows*

Cow No.	Cane silage group				Cow No.	Kafir silage group			
	Silage consumed	Calculated grain in silage	Grain recovered from manure	Loss		Silage consumed	Calculated grain in silage	Grain recovered from manure	Loss
	Pounds	*Pounds*	*Pounds*	*Per cent*		*Pounds*	*Pounds*	*Pounds*	*Per cent*
1	300	11.16	3.60	32.26	5 [a]	268	8.23	2.41	[a] 29.25
2	360	13.39	4.83	36.08	6	340	10.44	6.09	58.35
3	360	13.39	4.65	34.70	7	420	12.89	6.65	51.57
4	354	13.17	4.25	32.29	8	336	10.32	3.90	37.84
Total	1,374	51.11	17.33	33.91	Total, excluding cow 5	1,096	33.65	16.64	49.46

[a] Cow 5 refused to eat a part of the kafir silage for three days previous to and for four days during the experimental period. The manure voided, therefore, represents less than 268 pounds of kafir silage consumed during the 10-day period in which manure was collected.

The question naturally arose as to whether the cows had derived any benefit from the whole grain voided in the manure. By weighing 1,000 kernels, dried to constant weight, as obtained from silage and from the manure, it was found that the kafir grain had decreased 2.98 per cent in weight. Likewise the cane seed showed a decrease of 7.85 per cent in the weight of an equal number of kernels. All weights were of hull-free kernels.

Chemical analyses were made of the hull-free kernels to ascertain if possible what changes had taken place in the kernels during their passage through the digestive tract. The kafir and cane kernels recovered from the manure were found to have a greater ash content than those from silage, due possibly to absorption of certain salts excreted into the alimentary tract. The ether extract was decreased about one-fifth. Crude fiber, contained largely in the outer covering of the kernel, was reduced slightly in percentage in the kafir sample, but for some unexplained reason appeared to increase in the sample of cane seed. Perhaps this increase may be attributed to a somewhat greater loss of the contents of the cane seed. A very small loss in crude protein was observed in the cane and kafir kernels. The nitrogen-free extract was not significantly affected. The character and extent of these changes may be noted in Tables 2 and 3.

The proportion of hulls and the closeness with which they clasp the kernels varies with cane and kafir. The percentage of kafir-grain hulls obtained from the silage was 13.75 per cent and was reduced to 0.50 per cent as recovered from the manure. Likewise, the percentage of hulls on the cane seed was reduced from 5.77 to 4.20 per cent by passage through the cow's digestive tract.

TABLE 2.—*Composition of hull-free cane and kafir kernels as obtained from silage and from manure*

Sample	Moisture	Ash	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract
Kafir grain:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
From silage	10.25	0.81	9.88	1.66	74.17	3.23
From manure	10.40	1.54	9.36	1.44	74.61	2.65
Cane seed:						
From silage	10.56	.60	8.40	2.11	74.83	3.50
From manure	9.44	1.43	8.35	2.88	75.06	2.84

TABLE 3.—*Changes in composition of dry matter in hull-free cane and kafir kernels which occurred during passage of the silage through the digestive tract of the cow*

Item	Kafir kernels		Cane kernels	
	Actual change (grams)	Percentage change	Actual change (grams)	Percentage change
Crude protein	−0.60	−5.45	−0.17	−1.81
Ether extract	−.65	−18.06	−.78	−19.94
Nitrogen-free extract	1.24	1.50	.78	.94
Crude fiber	−.24	−13.04	.83	35.32
Ash	.82	91.11	.90	134.33
Total weight	−.436	−2.98	−1.002	−7.85

DISCUSSION OF RESULTS

The data presented show that approximately one-third of the cane seed and over two-fifths of the kafir grain in silage made from these plants, as used in this feeding experiment, were voided in the manure. A comparison of the chemical analyses of the grain from the silage and of that from the manure shows that a negligible quantity of the nutrients in these undigested kernels was utilized. The increased percentage of ash may be explained by the absorption of salts by the grain while in the digestive tract of the cow.

The losses of whole kernels were sufficient to raise the question again as to the desirability of heading cane and kafir before ensiling these crops. If the heads are first removed, they may be ground and added to the grain ration. If they are not removed but are fed in the silage, pigs or poultry may be given access to the manure to salvage a part of the lost grain.

The cost of labor and time, the facilities available, as well as the value of kafir grain as feed, are factors to be considered in deciding whether to head cane and kafir before putting these crops into the silo.

SUMMARY AND CONCLUSIONS

When dairy cows were fed cane and kafir silage made from fairly mature whole plants, one-third of the cane seed and over two-fifths of the kafir grain were voided in the manure.

Chemical analyses showed little utilization of nutrients from these whole kernels during passage through the cow's digestive tract. Some ether extract was digested, whereas only a small percentage of the crude protein was utilized. The effect upon the crude fiber was variable.

Heading cane and kafir silage before ensiling these crops is recommended. Such a practice will depend upon several economic factors, such as labor, facilities, and the value of kafir grain as feed.

When cows are fed silage made from the entire plant, the whole grain which passes out into the manure may be salvaged by pigs or poultry.

THE RELATION BETWEEN THE VITAMIN B CONTENT OF THE FEED EATEN AND OF THE MILK PRODUCED[1]

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INTRODUCTION

It has been clearly demonstrated by investigators in England (*1*, *3*)[3] and America (*5*, *7*, *8*, *9*, *10*) that the degree of potency of vitamins A and C in milk is largely dependent on the diet of the cow. The greater part of the evidence has been obtained from comparisons of milk produced on summer pasture with milk produced on the usual winter ration, consisting of silage, grain, and hay.

It would appear reasonable to expect also that the vitamin B growth-promoting factor in milk would be dependent on the diet of the cow. Evidence obtained by several investigators does not bear out such assumption in all instances. Osborne and Mendel (*11*) have reported that from the standpoint of vitamin B potency milk from pasture-fed cows was not superior to that from cows receiving winter rations. Later investigations were conducted by Kennedy and Dutcher (*10*), Hughes, Fitch, and Cave (*9*), who concluded that the presence of vitamin B in milk was dependent on the presence of this factor in the diet of the cow. More recently Hart (*6*) has expressed the opinion that milk produced by cows on pasture is no richer in vitamin B than that from cows fed the usual winter ration of grain, hay, and silage. It has also been reported that it is not possible through the feeding of excessive quantities of wheat germ to increase the vitamin B content of cow's milk (*12*, *p. 18*). Just recently Bechdel, Eckles, and Palmer (*2*) concluded that the feeding of cows on a ration deficient in vitamin B slightly reduced the vitamin B content of the milk. As the ration fed the cows used in this investigation was unpalatable and was not eaten in amounts sufficient for satisfactory nutrition, the authors were reluctant to consider the results as being highly significant.

For the past three years one of the authors has been in charge of an investigation to determine the vitamin B requirements of calves. Conclusive evidence has been obtained to show that a calf will grow normally to maturity, and produce normal offspring on a ration that carries an insufficient amount of vitamin B to support growth and well-being in rats (*2*). Since the animals used in these investigations had been fed on such a ration for over two years at the time of freshening, an excellent opportunity was afforded

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[2] The authors wish to acknowledge their appreciation of the helpful advice received from Prof. R. A. Dutcher in planning and carrying out the experiment.

[3] Reference is made by number (italic) to "Literature cited," p. 287.

for a study of the vitamin B content of milk which was produced on a ration definitely known to be deficient in vitamin B.

In all of the investigations previously carried out as cited above, the vitamin B content of the ration fed to the cows was not definitely determined through rat-feeding trials. Furthermore, in the previous investigations, the deficient ration was not fed for such a prolonged period of time before the cows began producing milk.

EXPERIMENTAL PROCEDURE

The object of the present study was to determine through laboratory feeding trials with rats, the vitamin B potency of milk produced by three of the vitamin B growth experimental animals (*2*), Nos. 1168, 1169, and 1170. Two of these cows, Nos. 1169 and 1170, had received the ration deficient in vitamin B (found so by 11 rat-feeding trials) throughout their growth and reproductive careers, a period of about 750 days. The other cow, No. 1168, had received a vitamin B supplement to her ration for a part of the time during her growth period (*2*, *p. 424*). Continued feeding of this vitamin B supplement (marmite yeast) was found unnecessary for growth and well-being, and it was dropped from the ration almost 300 days before she freshened.

Animal No. 1169 freshened December 18, 1925, and No. 1168 a week later. Two weeks after the latter had freshened, about 10 quarts of her milk and 30 quarts from No. 1169 were saved from 4 successive milkings for the experiments. The milk from these heifers was mixed, poured in sterile containers, sealed, and frozen solid. A refrigerator maintained at about 10° F. below zero was used for storing the milk until it was needed for experimental feeding.

The milk was all collected at one time for the reason that there was some doubt as to whether the heifers would be able to lactate on the experimental ration for any great length of time. It was fortunate that such provision was made, since it developed a few days later that the experimental ration was inadequate to meet the heavy demands of lactation. Subsequent investigations, the details of which will be submitted in a later paper, established definite proof that the apparent deficiency was not due to an inadequate supply of vitamin B. Since vitamin B storage has not been found in other species (*13*), it would have been very interesting to study this phase of the problem on cattle. The authors appreciated, however, that the possibility of vitamin B storage in these heifers was almost negligible, and also that the fact that the milk was obtained early in lactation would not materially depreciate the value of the data.

Animal No. 1170 freshened February 6, 1926, after it had been fed the deficient ration 776 days. Her milk was sampled and stored for experimental feeding as described above.

The experimental ration fed to the heifers was made up of dried sugar-beet pulp as the sole source of roughage and the following concentrates: Corn gluten, pearled hominy from white corn, polished rice, cornstarch, imported casein (Argentine), cane sugar, bone

meal, and a mineral mixture. Cod-liver oil was fed daily to supply fat soluble vitamins.[4]

The laboratory methods previously employed for vitamin studies by Dutcher and his associates (*4*) were used in this investigation. A vitamin B free basal ration (*4, p. 383*) was fed in connection with the milk, as the sole source of vitamin B supply. Twelve cubic centimeters of milk from cows receiving good winter rations when fed in this way had been found necessary to support normal growth. The experiments were planned, therefore, with five groups of rats in order to study daily allowances per rat of 8, 10, 12, 16, and 20 c. c. of milk. Twelve rats were allotted to each group at the beginning of the experiment, and the mixed milk from cows Nos. 1168 and 1169 was fed to them. Beginning with the ninth week of feeding, all of the rats but the group allowed 20 c. c. of milk daily were fed milk from heifer No. 1170, as the remaining supply from the other heifers was inadequate to complete the experiment.

The rats were put on experiment at an average age of 24 days. Their average initial weight was 37 gm. In all of the work individual cages with screen bottoms (3 meshes to 1 inch) were used, a practice which has been rigidly carried out in this laboratory for over three years. Iodine was fed in the drinking water. The milk was fed in a container separate from the basal ration, and two drops of cod-liver oil were added to the milk daily to insure an adequate supply of vitamins A and D.

DISCUSSION

The experimental data were averaged and put in graphic form as presented in Figure 1. The groups fed 8 and 10 c. c. of milk (curves A and B) deviate markedly from the normal. None of the individual rats in the group allowed 8 c. c. were normal in size at the end of 91 days of feeding, although every individual continued to gain in weight during the entire feeding period. Similar comment may be made also concerning the group allowed 10 c. c. of milk, except that one individual was normal in size at the end of the experiment. There was apparently no tendency toward pathological conditions in any of the rats of the two groups above mentioned or of the groups fed larger quantities of milk.

The group receiving 12 c. c. of milk (curve C or curve H) made much better growth than those receiving less. Nine rats, or 50 per cent of this group, were of normal or larger size at the end of 84 days of feeding.

When the feeding of milk from heifer No. 1170 was started as mentioned above, an additional group of six rats was put on an experiment in which 12 c. c. of milk was added to the basal ration. This was done in order to determine whether the growth of the young, more rapidly growing animals would indicate any difference in the milk from another cow on the same diet. No detectable differences in the growth of any of the groups of rats was observed when the milk was changed. However, the growth of the additional six animals conformed closely to that of the rats from the same stock

[4] Details concerning the ration and feeding technic may be found in the Journal of Dairy Science (*2, p. 417*).

that were fed by Dutcher, Francis, and Combs (*4*) on 12 c. c. of raw milk from the college herd. For convenience of study, a copy of the growth curve on the latter data has been placed on the chart (curve F) beside those of the two groups of rats fed 12 c. c. of raw milk in this experiment (curves G and H). (Curve F copied from Dutcher, Francis, and Combs.)

In order to have enough animals of one age and weight to start the five groups at one time, the rats used were purchased from a supply company. It so happened that they were of a strain of a somewhat lighter weight than the laboratory stock. It is entirely probable that their growth (curve H) is as near normal for their weight as that of the Pennslyvania State Stock (curve G). From the evidence presented by this additional group in connection with

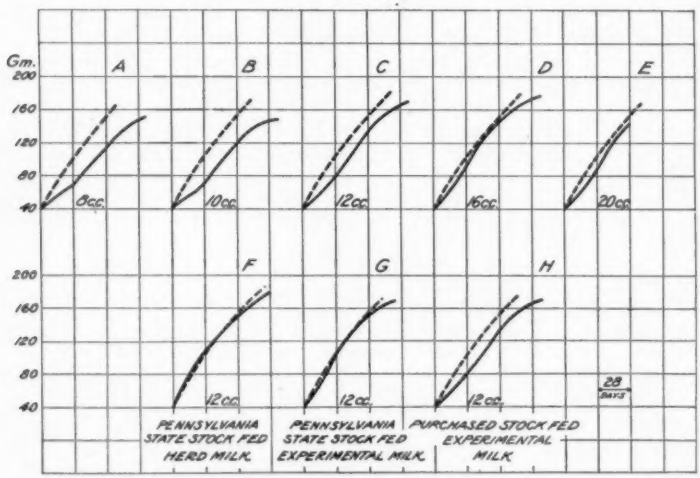

FIG. 1.—Growth curves. Broken lines represent normal growth of rats. Unbroken lines represent average growth of groups of rats fed different amounts of the experimental milk (excepting curve *F*, as indicated) as a supplement to a basal ration

that of the original 12 rats, it is apparent that 12 c. c. of milk from cows on a ration deficient in vitamin B carried as much vitamin B as 12 c. c. of milk from cows receiving good winter rations.

The groups of rats allowed 16 c. c. (curve D) and 20 c. c. (curve E) of milk were so nearly identical that the experiment with the latter was discontinued after 56 days in order to save the milk for further feeding. Had the rats in these groups been of the Pennsylvania State stock their growth curve would no doubt have been above the Donaldson normal curve which is used in presenting all data from this laboratory. Furthermore, had the Pennsylvania State stock been used in the groups fed 8 and 10 c. c. of milk, the growth curves would have approached more closely to the normal.

The authors have for some time been conducting experiments to determine the possible synthesis of vitamin B by bacterial fermentations in the rumen of the digestive tract, as mentioned in an earlier

paper (*2*). Although these experiments are not completed, it may be said at this writing (April, 1927) that some very positive evidence has been obtained.

SUMMARY

This investigation was conducted to determine the vitamin B potency of milk from three cows that were fed for over two years, throughout their growth period, on an experimental ration that was decidedly deficient in vitamin B growth factor. The milk was fed to rats as a supplement to a vitamin-B-free basal ration on levels of 8, 10, 12, 16, and 20 c. c. per rat per day. The vitamin B potency of the milk was found equal to that of herd milk from cows receiving a good winter ration. It is concluded that vitamin B in milk is not dependent upon the presence of this vitamin in the ration of the cow. It would appear that cattle, and possibly all ruminants, differ from other animals in their ability to grow to maturity, to produce normal offspring, and to maintain vitamin B in their milk when forced to subsist on rations deficient in vitamin B. If this is true, it would appear that ruminants possess the power to synthesize vitamin B. Experiments are in progress to determine whether this synthesis is due to microorganisms normally present in the rumen.

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